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Different responses to environmental factors in terpene composition of *Pinus heldreichii* and *P. peuce*: ecological and chemotaxonomic considerations

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Abstract: Many studies show the influence of the environment on terpene composition, but not many of them deal with the terpene composition variability in correlation with environmental factors in *Pinus*. We chose two endemic species – *P. heldreichii* Christ. (Bosnian pine) and *P. peuce* Griseb. (Macedonian pine) – two relict and (sub)endemic species of the Balkan peninsula. They mainly form pure stands but can appear in mixed populations, most commonly with each other. These species belong to different subgenera and thus differ greatly, especially in needle anatomy. α-pinene, limonene and germacrene D predominated in Bosnian pine leaf oleoresins, while Macedonian pine oleoresin contained high amounts of α-pinene. Furthermore, there were three chemotypes present in Bosnian, and only one in Macedonian pine. Oleoresins of Bosnian pine did not show correlation with climate, however, its composition changed in response to geological substrate type. Macedonian pine oleoresin showed a high correlation with the climate but changed only a little in response to geological substrate type. The oleoresin profiles showed strong species-dependent composition and variability. However, since each species expressed different responses to tested environmental conditions, it is important to take into consideration these variables when analyzing natural populations of the species.

Keywords: Bosnian pine; Macedonian pine; essential oil; environmental factors; chemotaxonomy

INTRODUCTION

Plants produce a myriad of compounds in order to adapt as best as they can to the environment. Essential oils or plant volatiles are complex mixtures consisting mainly of terpenes (mono-, sesquiterpenes and some diterpenes), but also different aliphatic and aromatic hydrocarbons. Essential oils play a significant role in plant-environment interactions, including both biotic (microorganisms, animals, plants, and fungi) and abiotic (climate and geological substrate) factors [1-3]. Terpenes are synthesized in two different cellular compartments: monoterpenes and diterpenes in plastids (MEP biosynthetic pathway) and sesquiterpenes in the cytosol (MVA) [3]. Their synthesis is regulated by terpene synthases that can produce anywhere from only one compound to up to 52 different compounds [4-10]. The multi-product synthesis of terpene synthases and a very fast rate of evolution of their genes is in high correlation with the ecological significance of the terpenes and the cost of their production [7,10-12]. Hence, terpenes are most useful at lower taxonomic levels. In conifers, essential oils are usually mixed with higher terpenes, and thus are referred to as oleoresins. They have been used as environmental and chemotaxonomic markers in many conifer species, with the latter ranging from family to infra-specific level [13-20]. Unlike some members of the Cupressaceae family, oleoresins in *Pinus* are stored in resin ducts located in leaves and branches, where they are used as protection against predatory insects [21].

There are many studies showing the influence of environmental factors on terpene composition [22-25]. Even though there are quite a few studies of the composition of the *Pinus* oleoresins, not many of them deal with the variability of their composition in correlation with environmental factors [26-28]. Furthermore, there are no studies on the influence of the environmental factors or their correlation with

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the composition of needle oleoresins of several Balkan pines.

For this study, we chose two endemic species – *Pinus heldreichii* Christ. (whitebark pine or Bosnian pine) and *P. peuce* Griseb. (Macedonian pine). Both species are relict and endemic or subendemic (*P. heldreichii*) on the Balkan peninsula. They can be found in high mountains of Serbia, Montenegro, Albania, Macedonia, Bulgaria and Greece, while Bosnian pine can also be found in southern Italy and Bosnia and Herzegovina [29]. These species usually form pure stands, but can also appear in mixed population, most commonly with each other. Furthermore, these two species belong to different subgenera within the genus. Bosnian pine belongs to the subgenus *Pinus*, while the Macedonian pine belongs to *Strobus,* differing greatly, especially in the anatomy of needles. Initial research on the distribution of these two species in their natural range suggested that they have a preference for different substrata, but consequent studies showed that they grow on both geological substrates [30,31]. This, along with their other characteristics, makes them perfect for this study.

The aims of this study were to assess the overall variability of oleoresin composition of two endemic pines from central Balkans in relation to their different geographic distribution and bioclimatic parameters, geological substrate and exposition.

MATERIALS AND METHODS

Plant material

Two-year-old twigs with needles were collected in late summer from 2003 and 2012. Plant material was collected from the lowest third of the tree crown, from 30 individuals in most populations, except Ošljak and Galičica, where there were small populations and therefore fewer samples were taken. Geographic and geological details of the studied localities and a map with the geographic distribution of the analyzed populations are given in the supplementary material (Supplementary Fig. S1; Supplementary Table S1). The collected twigs were stored in a freezer (-21°C) until oleoresin extraction and analysis.

Oleoresin isolation

Two-year-old needles were cut into 2-3-mm pieces and extracted with GC grade *n*-pentane (1 g of needles per mL of solvent). The extracts were kept at 4°C for 24 h, then filtered and stored in chromatography vials with solid caps in a refrigerator until GC/MS analyses.

GC/MS and GC/FID analyses

The GC/MS analyses were performed with a Hewlett Packard G1800C-GCD apparatus equipped with a split/splitless injector, an automatic liquid sampler (ALS), a mass-selective detector and an HP-5 MS fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 mm). The chromatographic conditions were the same as described (cf. GC-FID Analysis), except for the carrier gas, which was He. Electron-impact mass spectra (EI-MS; 70 eV) were acquired over the m/z range 40-450 amu.

The GC-FID analyses were carried out with an Agilent Technologies 7890A apparatus equipped with a split/splitless injector, an ALS, a flame ionization detector (FID) and an HP-5 fused-silica capillary column. The oven temperature was programmed linearly to rise from 40 to 280° at 4/min; injector temperature was 250°C; detector temperature was 280°C; carrier gas, H2 (1 mL/min). Samples (1 mL) were injected in splitless mode (1:15) using the ALS.

Bioclimatic parameters

Bioclimatic characteristics of the nine studied localities were extracted from the WorldClim set of global climate layers [32] using DIVA-GIS 7.5 software (available at: http://www.Diva-Gis.Org). The list of used abbreviations for bioclimatic parameters (BIO1-19) is given in Supplementary Table S4.

Statistical methods

Multivariate analyses, discriminant analysis (DA) and hierarchical cluster analysis (HCA) were performed using PAST ver. 3.21. [33]. The simple correlation test, as well as the Mantel and partial Mantel tests, were used to evaluate the correlations with bioclimatic parameters, geological substrate type, and terrain exposition.

RESULTS

Pinus heldreichii

In total, 138 compounds were detected. The detailed oleoresin composition of the studied individuals can be found in our previous investigations [34,35]. Twenty components that did not show correlation and were present at 0.5% on average in the whole dataset were used for further statistical analyses (Table 1). These twenty compounds represented on average 90.2±4.3% of the total oil content. Even though essential oil composition varied between individual samples, three components were always high in abundance in all of them, namely, *α*-pinene, limonene and germacrene D. Limonene was the most abundant compound in most of the individual samples in all individuals from the population Galičica, and most individuals from the populations Lovćen, Zeletin, Bjelasica and Revuša, and a single individual from the population Ošljak. On the other hand, germacrene D was the dominant component of almost all individuals from Ošljak and several other populations excluding Galičica. Only 10 individuals (out of 144) from Lovćen, Zeletin, Bjelasica and Revuša had *α*-pinene as a dominant compound.

DA based on these components with populations as groups showed the formation of three clusters. Based on the high abundance of *δ*-3-carene and low abundance of *α*-pinene, the population Revuša separated on one side. On the other side, the populations Ošljak and Galičica parted based on the high abundance of germacrene D, while all other populations overlapped based on the lower abundance of germacrene D and *δ*-3-carene, and the relatively high abundance of *α*-pinene and *ß*-caryophyllene (Fig. 1a). These results were further confirmed by cluster analysis (Fig. 1b).

A linear correlation test was used to assess the potential correlation between essential oil composition and bioclimatic characteristics, altitude and surface inclination. The linear correlation test showed a weak correlation between several essential oil components and several bioclimatic parameters, mainly those related to precipitation (Supplementary Table S2). However, there was no correlation between terrain

Table 1. Relative abundance of twenty compounds of *Pinus heldreichii* essential oil used in statistical analyses.

	Compound[a]	Galičica (n=8)[b]	Ošljak (n=16)	Lovćen (n=30)	Zeletin (n=30)	Bjelasica (n=30)	Revuša (n=30)
1	*trans*-2-Hexenal	0.3 ± 0.2	0.6 ± 0.4	0.6 ± 0.4	2.0 ± 1.5	2.6 ± 2.3	0.1 ± 0.1
2	**α-Pinene**	**22.2 ± 3.7**	**13.2 ± 4.8**	**20.3 ± 10.1**	**17.3 ± 6.4**	**16.1 ± 4.1**	**11.1 ± 8.9**
3	Camphene	1.2 ± 0.2	0.7 ± 0.2	0.9 ± 0.4	1.1 ± 0.3	1.1 ± 0.5	0.5 ± 0.3
4	*ß*-Pinene	7.2 ± 1.6	4.2 ± 1.8	6.5 ± 2.1	6.0 ± 2.7	4.8 ± 1.4	3.6 ± 3.0
5	*ß*-Myrcene	2.8 ± 0.3	2.1 ± 0.2	2.2 ± 0.6	2.5 ± 0.5	1.8 ± 0.4	2.3 ± 0.7
6	*δ*-3-Carene	-	-	-	0.2 ± 1.2	-	8.5 ± 10.8
7	**Limonene**	**31.9 ± 2.9**	**24.7 ± 3.6**	**25.1 ± 9.8**	**29.8 ± 6.6**	**23.2 ± 4.7**	**24.1 ± 13.4**
8	*α*-Terpinyl acetate	0.4 ± 0.2	0.2 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.3 ± 0.2	0.7 ± 0.4
9	*ß*-Caryophyllene	4.5 ± 2.0	8.1 ± 1.8	8.9 ± 2.1	11.4 ± 2.4	11.0 ± 2.8	9.7 ± 2.5
10	Calarene	0.3 ± 0.0	0.4 ± 0.1	1.6 ± 0.4	1.2 ± 0.6	1.4 ± 0.5	-
11	Aromadendrene	-	-	0.9 ± 0.2	0.7 ± 0.4	0.9 ± 0.3	0.1 ± 0.0
12	*α*-Humulene	0.8 ± 0.3	1.4 ± 0.2	1.9 ± 0.4	2.2 ± 0.5	2.2 ± 0.5	2.3 ± 3.9
13	*γ*-Muurolene	0.1 ± 0.1	0.1 ± 0.1	1.2 ± 0.3	1.0 ± 0.6	1.1 ± 0.5	0.2 ± 0.4
14	**Germacrene D**	**22.0 ± 2.9**	**32.1 ± 6.9**	**15.4 ± 4.9**	**11.4 ± 6.2**	**13.1 ± 6.5**	**21.2 ± 9.2**
15	*α*-Muurolene	0.3 ± 0.1	0.3 ± 0.0	1.6 ± 0.5	1.5 ± 0.9	1.8 ± 0.9	0.2 ± 0.1
16	*γ*-Cadinene	0.1 ± 0.0	0.2 ± 0.0	1.0 ± 0.3	0.8 ± 0.5	0.9 ± 0.4	0.1 ± 0.1
17	*δ*-Cadinene	0.2 ± 0.0	0.4 ± 0.1	1.7 ± 0.6	1.5 ± 0.9	1.9 ± 0.8	0.3 ± 0.1
18	Germacrene D 4-ol	0.4 ± 0.1	0.8 ± 0.3	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.3
19	Methyl arachidonate	-	-	0.6 ± 0.8	0.5 ± 0.2	0.4 ± 0.3	0.9 ± 0.3
20	Isopimarol	0.5 ± 0.2	0.6 ± 0.3	1.1 ± 0.5	1.1 ± 0.7	1.0 ± 0.6	2.2 ± 1.1

[a] Contents of the essential oil composition are given as percentages (mean±SD); -: not detected; [b] n – number of individuals in each studied population is given in parentheses; for detailed information on geographic and geologic characteristics of the investigated localities, *cf. Supplementary Table S1*.

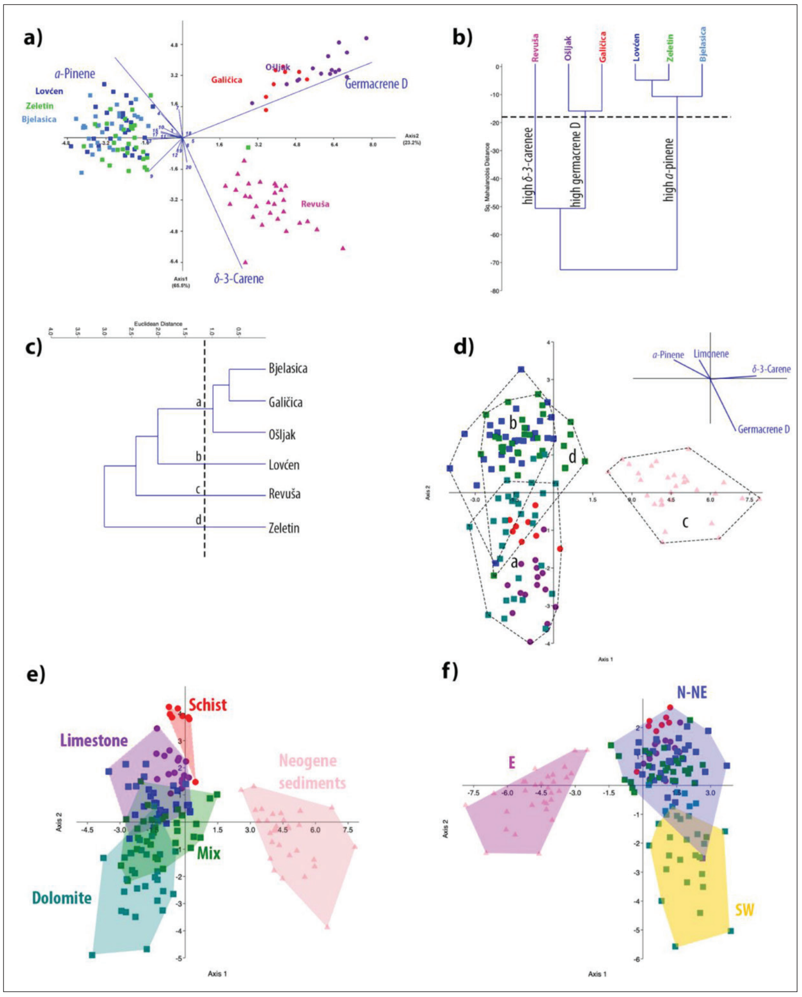

Fig. 1. a – DA based on 20 components of essential oil; ● – Ošljak, ● – Galičica, ▲ – Revuša, ▪ – Bjelasica, ▪ – Lovćen, ▪ – Zeletin; **b** – cluster analysis based on 20 components of essential oil (Sq. Mahalanobis distance, UPGMA); **c** – HCA analysis based on the 19 bioclimatic parameters of studied localities; **d** – DA scatter plot based on 20 essential oil components, group responses to bioclimatic groups from HCA; **e** – DA scatter plot based on 20 essential oil components, grouped according to geological substrate; **f** – DA scatter plot based on 20 essential oil components, groups according to population exposition.

inclination or altitude with any of the twenty essential oil compounds. Calarene, aromadendrene, γ-muurolene, δ-cadinene and γ-cadinene showed a moderate positive correlation with several bioclimatic parameters related to precipitation, although this could be due to the very low abundance of these compounds. None of the dominant essential oil components showed correlation with any of the used bioclimatic parameters. The Mantel and partial Mantel tests showed no correlation between the essential oil profile and bioclimatic parameters, not even after the removal of the geographic region. To further assess the possible influence of climate, exposition and substratum, DA was deployed. Groups for DA were constructed using bioclimatic parameters, exposition and geological substrate, respectively, to assess whether the populations growing in different conditions separate significantly based on essential oil components.

Based on the bioclimatic characteristics of the localities, four groups could be distinguished (Fig. 1c). Bjelasica, Galičica, and Ošljak formed one group (*a*), while all other populations belonged to different groups (*b*-*d*). These bioclimatic groups differ in minimum winter temperatures, with group *d* having the coldest winters, followed by groups *c, a* and *b*. Group *c* (Revuša) also had quite a different climate, with the highest temperatures during the wettest quarter and lowest during the driest. However, the DA scatterplot shows a high separation of only Revuša (bioclimatic group *c*) based on the high abundance of δ-3-carene and complete overlap of all other bioclimatic groups.

DA was performed with groups according to geological substrate type and exposition. Individuals growing on Neogene sediments separated from individuals growing on limestone-based substrates (Fig. 1e). Schist and limestone substrate populations separated based on the higher abundance of α-pinene and limonene, while dolomite populations separated based on β-caryophyllene. Interestingly, individuals from a population that grew on a serpentinite substrate were positioned between populations growing on limestone and dolomite substrates. The DA scatterplot with individuals growing at different expositions showed complete overlap of individuals growing on N and E expositions (Figure 1f). These individuals had a higher abundance of δ-3-carene and a lower presence of α-pinene. On the other hand, the population growing with a NE exposition had a higher

Table 2. The relative abundance of eleven compounds of *Pinus peuce* essential oil used in statistical analyses.

		Ošljak	**Pelister**	**Zeletin**	**Sjekirica**	**M. Gora**
	Compound[a]	(n=19)[b]	(n=30)	(n=30)	(n=30)	(n=30)
1	***α*-Pinene**	**45.4 ± 4.0**	**45.6 ± 3.7**	**35.3 ± 2.6**	**36.9 ± 3.2**	**37.4 ± 4.2**
2	**Camphene**	**10.7 ± 3.3**	**10.1 ± 2.3**	8.7 ± 1.0	8.4 ± 1.5	8.5 ± 2.5
3	***β*-Pinene**	8.6 ± 2.6	**12.1 ± 4.9**	7.1 ± 2.3	4.6 ± 4.0	8.7 ± 3.4
4	*β*-Myrcene	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
5	*β*-Phellandrene	3.7 ± 0.9	3.1 ± 0.6	4.7 ± 0.9	4.7 ± 1.3	4.7 ± 0.6
6	Bornyl acetate	5.7 ± 2.1	4.5 ± 1.4	7.5 ± 1.9	6.8 ± 1.8	6.2 ± 1.9
7	Terpinen-4-ol acetate	-	-	1.6 ± 0.9	1.6 ± 1.0	1.5 ± 0.7
8	*α*-Terpinyl acetate	0.9 ± 0.4	0.8 ± 0.3	1.0 ± 0.4	0.9 ± 0.3	0.9 ± 0.5
9	*α*-Humulene	0.5 ± 0.1	0.4 ± 0.1	-	1.1 ± 0.2	1.1 ± 0.1
10	**Germacrene D**	**11.1 ± 3.3**	**11.1 ± 2.0**	**11.2 ± 2.9**	**12.0 ± 2.9**	**10.9 ± 2.5**
11	Germacrene D-4-ol	1.4 ± 2.7	0.6 ± 1.1	0.5 ± 0.5	0.5 ± 1.2	0.4 ± 0.3

[a] Contents of the essential oil composition are given as percentages (mean ± SD); -: not detected; [b] n – the number of individuals in each studied population is given in parentheses; for detailed information on geographic and geologic characteristics of the investigated localities, *cf. Supplementary Table S1.*

abundance of germacrene D and was clearly separated from all other populations. The population with a SW exposition had the highest abundance of *α*-pinene and low abundance of both *δ*-3-carene and limonene.

Pinus peuce

In total, 135 compounds were detected. The detailed oleoresin composition of the studied individuals can be found in our previous investigations [36,37]. Eleven that did not exhibit correlation and were present at 0.5% on average in the whole data set were used for further statistical analyses (Table 2). These eleven compounds represent on average 84.1±5.0% of the total oil content. Even though the essential oil composition varied between individual samples, *α*-pinene was dominant in all samples (29.3-55.5%). Several other compounds showed high abundance, i.e. camphene, *β*-pinene, bornyl acetate, germacrene D. One individual from Ošljak also had germacrene D-4-ol in relatively high abundance.

DA based on these components with populations as groups showed the formation of three clusters. All individuals separated primarily on the abundance of the two most abundant compounds (*α*-pinene and *β*-pinene), however, they separated clearly based on several other compounds on the first discriminant axis. The population Zeletin separated from all others based on the absence of *α*-humulene. On the other hand, populations Ošljak and Pelister did not have terpinen-4-ol acetate and had quite a low abundance of *α*-humulene (<1%). The populations Sjekirica and Zeletin contained both *α*-humulene and terpinen-4-ol acetate at a somewhat higher abundance (Fig. 2a). These results were further confirmed by cluster analysis (Fig. 2b).

A linear correlation test was used to assess the potential correlation between essential oil composition on one side and bioclimatic characteristics as well as altitude and inclination of the surface (Supplementary Table S3). A strong significant correlation was found of *α*-pinene and terpinen-4-ol acetate with bioclimatic parameters and altitude. There was no correlation between terrain inclination with any of the analyzed essential oil components. *α*-Pinene showed a medium to strong positive correlation with bioclimatic parameters related to temperature (BIO1-BIO11), and a strong negative correlation with bioclimatic parameters related to precipitation and altitude, with the exception of precipitation seasonality (BIO15). The opposite holds true for terpinen-4-ol acetate. This compound displayed a medium negative correlation with bioclimatic parameters related to temperature and precipitation seasonality, and a positive correlation with the precipitation parameters. The Mantel and partial Mantel tests showed a high statistically significant correlation between the essential oil profile and bioclimatic parameters (r=0.95 p=0.01), even after the removal of the geographic region (r=0.91, p=0.03), which also strongly correlated with the essential oil profile (r=0.72, p=0.02). To further assess the possible influence of the climate, exposition and substratum,

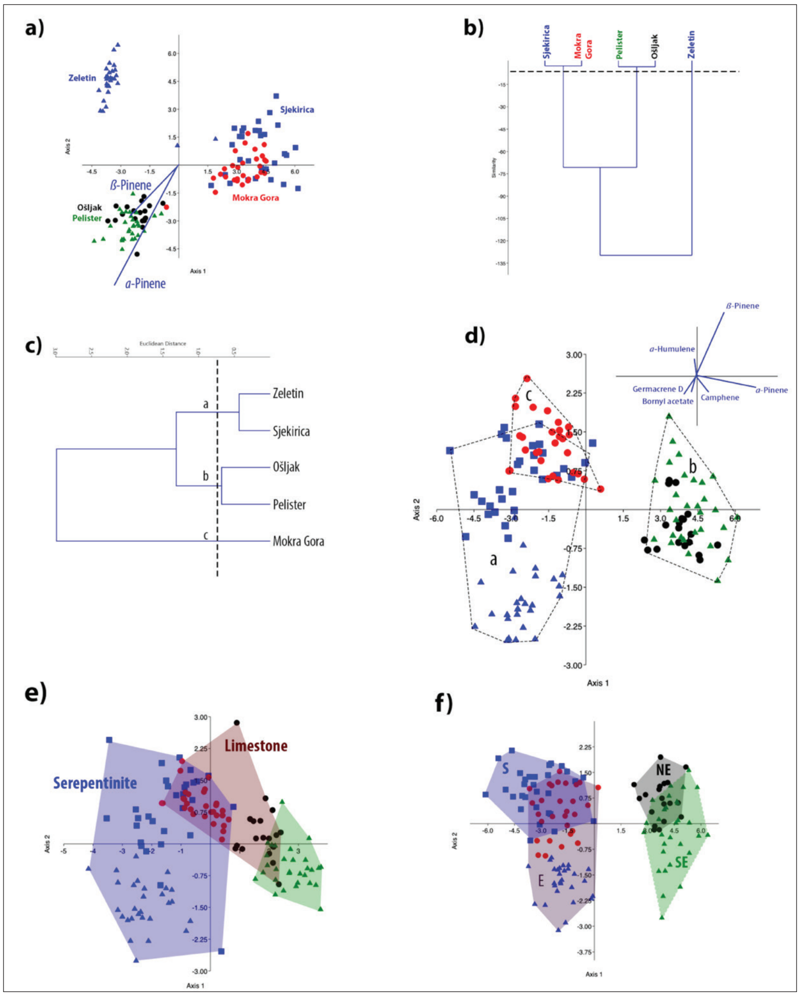

Fig. 2. a – DA based on 11 components of essential oil; ▪ – Sjekirica, ● – Mokra Gora, ▲ – Pelister, ● – Ošljak, ▲ – Zeletin; **b** – cluster analysis based on 11 components of essential oil (Sq. Mahalanobis distance, UPGMA); **c** – HCA analysis based on the 19 bioclimatic parameters of studied localities; **d** – DA scatter plot based on 11 essential oil components, group responses to bioclimatic groups from HCA; **e** – DA scatter plot based on 11 essential oil components, grouped according to geological substrate; **f** – DA scatter plot based on 11 essential oil components, groups according to population exposition.

Ošljak and Pelister (bioclimatic group *b*) based on the high abundance of *α*-pinene and almost complete overlap of all other bioclimatic groups. Mokra Gora (bioclimatic group *c*) separated from most of the individuals belonging to bioclimatic group *a*, based on the presence of *α*-humulene and higher abundance of *β*-pinene.

DA was also performed with groups according to geological substrate type and exposition. Even though there is overlap between substrate types, there is an evident gradient of both *α*-pinene and *β*-pinene along the pH gradient, from an ultra-alkaline serpentinite substrate to a less alkaline silicate (Fig. 2e). The DA scatter plot with groups based on locality exposition showed the formation of two separate clusters. One cluster was with individuals growing on NE and SE slopes, while the other one included those growing on S and E exposition. The NE-SE group separated based on the higher amounts of *α*-humulene, germacrene D-4-ol and camphene, while the S-E group had a higher abundance of bornyl acetate, *β*-phellandrene, germacrene D and terpinen-4-ol acetate (Fig. 2f). Interestingly, S and NE populations had similarly high amounts of *α*-pinene, while the E and SE populations had *β*-pinene.

and to identify components most affected, DA was performed. Groups for DA were constructed using bioclimatic parameters, exposition and geological substrate.

Based on the bioclimatic characteristics of the localities, three groups could be distinguished (Fig. 2d). Most distinct of all was Mokra Gora that was in a group of its own (*c*). Four other localities have much more similar climates, so they clustered close to each other, forming two clades: clade a (Zeletin and Sjekirica) and clade b (Ošljak and Pelister). However, the DA scatterplot shows a high separation of only

DISCUSSION

Bosnian and Macedonian pine, which belong to different subgenera, show remarkably different responses to environmental factors. The evident differences in essential oil profiles between the two pine species, even when growing in the same locality (Ošljak and Zeletin) under the influence of the same environmental factors, can only be explained by strong genetic control. This is in agreement with previous investigations on the usability of oleoresin/essential oil as chemotaxonomic markers in conifers (18,38-45). However, within the investigated species, there is a species-specific composition response to the environment.

There was no simple correlation between environmental factors and the composition of the essential oil in Bosnian pine. There are several chemotypes present in each of the populations, and the distribution of these chemotypes appears to be random. Based on the essential oil profile, three groups could be distinguished that correspond to geographic regions. However, the essential oil profile showed no correlation with bioclimatic parameters in any of the tests. Since 50-year averages were used for bioclimatic parameters, these results suggest that there was no shift in the essential oil profile that would correspond to the climate of the area; however, considering the lifespan of these individuals, this only confirms a strong genetic control, independent of fluctuating climate. More detailed experiments measuring real-time bioclimatic parameters and essential oil composition are needed to confirm this hypothesis.

On the other hand, population Revuša separated from all other populations based on the geological substrate type (Neogene sediments vs. limestone-based substrates). The exposition also showed some influence on the essential oil profile in Bosnian pine, separating individuals growing on the NE exposition, and a partial overlap of individuals from N, E and SW expositions.

Unlike the Bosnian pine, the Macedonian pine essential oil profile showed a high correlation with bioclimatic parameters, both in univariate and multivariate tests. DA showed the separation of bioclimatic groups with partial overlap of bioclimatic groups *a* and *c*, which would indicate that the influence of bioclimatic factors is not as simple as the test suggests. Considering the type of data used (50-year average), this indicates that either the individuals with better-suited oleoresin profiles were selected or that climate modulated more strongly the essential oil composition in this species. Geological substrate type also showed separation with some overlap of groups growing on different substrate types, from ultra-alkaline to less alkaline, but unlike Bosnian pine, there is no simple (e.g. linear) influence of exposition since the populations with similar exposition grouped opposite to one another.

The different responses of these two pines could be due to their different anatomy and physiology. Bosnian pine needles are significantly more robust and thicker than those of Macedonian pine. Furthermore, the epidermis of Bosnian pine needles and the cuticular layer are significantly thicker, providing both the photosynthetic tissue and vascular bundle with much better protection from drying-out or freezing. The resin ducts in which essential oil is produced and stored are much closer to the outer leaf surface in Macedonian pine than in Bosnian pine [46,47], so their oleoresins could play much more significant roles in leaf physiology and ecology than in Bosnian pine. Furthermore, the essential oil profile of Macedonian pine is much simpler than that of Bosnian pine, especially when we take into account that there is only one chemotype present in *P. peuce*.

This varying response is congruent with the scarce literature data available on the correlation of different abiotic factors and essential oil composition. For example, in an evergreen shrub *Rosmarinus officinalis* L., bioclimatic parameters related to the temperature were the dominant abiotic factors that led to the chemical differentiation of studied populations [22]. Marčetić et al. [23] showed that climate also had a high impact on the composition of the essential oils of different plant parts (roots, aerial parts, and fruits) of the perennial angiosperm, *Seseli rigidum* Waldst. & Kit., but the response of each plant part was different. Interestingly, the influence of the substrate was less pronounced. On the other hand, in another conifer species, *Juniperus communis* L., two studies present somewhat conflicting results [24,25]. One showed that the substrate characteristics and insolation did not influence the amount of α-pinene (a dominant component in *J. communis* leaf and berry essential oil) and its enantiomeric composition. The other study showed no influence of climate on the amount of the dominant component (α-pinene), but also revealed that several components of essential oil were influenced by the climate; the relative abundance of several components (humulene, ß-elemene, caryophyllene, α-terpinolene, and terpinen-4-ol) increased gradually from the coast to inland. These results further confirmed the complex relationship between plant anatomy, physiology, essential oil composition and environmental factors.

CONCLUSIONS

The essential oil profiles of Bosnian and Macedonian pine show species-dependent variability. Even when growing at the same locality under the influence of the same environmental factors, both species displayed very different essential oil profiles. However, the influence of environmental factors cannot be completely ruled out. In the more robust Bosnian pine needles, the essential oil composition was only mildly influenced by terrain exposition and geological substrate type, but did not show any correlation with differing climate conditions at the localities. On the other hand, in the more lithe needles of Macedonian pine, the oil profile was highly influenced by climate, but only mildly by substrate type. Therefore, while the essential oil profile in pines is genetically determined, one must take into account the geological substrate type, the climate and even the exposition when analyzing natural populations and chemophenetics of the species.

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Author contributions: NR, PM drafted the manuscript; BN and PM collected the plant material; BN conducted essential oil isolation and analyses; NR performed the statistical analyses; PM supervised the research and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/Rajcevic%20et%20al_4458_Supplementary%20Material.pdf