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*Original Scientific Paper*

# Terpene relationships among some soft and hard pine species

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**ABSTRACT:**
In this study we have reported the chemical composition of the essential oils obtained from the needles of twelve *Pinus* taxa, belonging to the subgenera *Pinus* (sections *Pinus* and *Trifoliae*) and *Strobus* (sect. *Quinquefoliae*). Monoterpenes dominated over sesquiterpenes in most of the investigated taxa of both subgenera, except in representatives of the subsection *Pinaster*, subgenus *Pinus*. *α*-Pinene was the most abundant terpene in eight pines, but other dominant terpenes were found in some taxa - *trans*-caryophyllene, germacrene D, abietadiene and *β*-pinene. In the subsect. *Pinaster*, the most dominant compounds varied from species to species. In addition, *P. halepensis* and *P. pinaster* had the highest percentage of diterpenes, while *P. heldreichii* was the richest in germacrene D. In the PCA and cluster analyses, three species from the subsect. *Pinaster* emerged as the most distant: *P. halepensis*, *P. pinaster,* and *P. heldreichii.* In addition, the possible taxonomic implications of the terpene profile in the analysed *Pinus* taxa were also discussed.

**Keywords:**
*Pinus*, diversity, principal component analysis, canonical discriminant analysis, cluster analysis

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## INTRODUCTION

*Pinus* L. is the largest genus of conifers and its approximately 110 species comprise ca. 50% of the Pinaceae family (Farjon 2001). The natural distribution of the genus is limited to the Northern Hemisphere with the exception of one population of *P. merkusii* situated south of the equator in Sumatra (Mirov 1967). Although classification differs among authors, the genus is usually divided into two subgenera: *Strobus* (Haploxylon or soft pines, with one fibrovascular bundle in the needles) and *Pinus* (Diploxylon or hard pines, with two fibrovascular bundles) (Little & Critchfield 1969). However, there are numerous inconsistencies in different studies regarding the number of sections and subsections as well as the position of several rare endemic pine species. In this paper we follow the classification proposed by Gernandt *et al.* (2005) who used chloroplast DNA trees and evidence from nuclear ribosomal DNA as well as morphology in order to propose a classification for this genus.

In addition to other specialized metabolites, terpenes are frequently used as chemosystematic markers in plants. Furthermore, the significance of terpenes and their effectiveness as genetic markers in analyses of biodiversity, geographic variability, evolution, and systematics in Pinales have been addressed in detail (Hanover 1992). The previously widely held opinion was that their biosynthesis is mainly under genetic control and not significantly influenced by environmental factors (Baradat & Yazdani 1988). On the other hand, many new studies have shown a certain variability in terpene composition, caused by various exogenous and endogenous factors such as the phases of the plant's ontogenetic development, type of organ or tissue, ecological factors, plant material processing procedures, and specific terpene isolation methods (Wang *et al.* 2014; Lee & Yee 2016 and the references cited therein; Nikolić *et al.* 2019; Rajčević *et al.* 2019).

Over the past two decades, there has been an increased interest in studying the chemical composition, as well as the biological activities of the essential oils isolated from different pine species (Ioannou *et al.* 2014; Mitić *et al.* 2018). The pronounced genetic diversity observed in the genus *Pinus* was reflected in its biochemical variability, usually studied at the level of terpene markers and isoenzyme variation (Ioannou *et al.* 2014 and references cited therein).

In this study we reported the chemical composition of the terpenes obtained from the needles of twelve *Pinus* taxa belonging to different subsections and sections of the subgenera *Pinus* and *Strobus*. In addition to sharing a common area (the Balkans), the specific terpene content of the analysed taxa also reflects the specific and unique local conditions (original geographical provenance), making such populations potentially important resources for phytopharmacy, cosmetics, and health and spa tourism.

The aim of this research is to gain insight into the terpene diversity in representatives belonging to different subsections and sections, keeping in mind that a rather limited number of species were reviewed to draw any solid taxonomic conclusion. The analysed taxa of the genus *Pinus*, be they autochthonous or allochthonous, exchange genetic material (gene pool) at the same time and in the same area (the Balkans), one of the world's important centres of biodiversity.

## MATERIALS AND METHODS

**Plant material.** Twigs with needles were collected from the lowest third of a full tree crown and stored at -20°C until the analyses of the needles were performed. The collection localities were as follows: parks in the city of Belgrade (Serbia) (*Pinus mugo*, *P. nigra* subsp. *nigra*, *P. sylvestris*, *P. heldreichii* and *P. ponderosa*, PMU, PNI, PSI, PHE and PPO, respectively), the Jevremovac Botanical Garden in the city of Belgrade (Serbia) (*P. peuce, P. strobus* and *P. wallichiana*, PPE, PST and PWA, resp.), and the island of Korčula in Croatia (*P. nigra* subsp. *dalmatica*, *P. halepensis*, *P. pinaster* and *P. pinea*, PND, PHE, PPN and PPI, resp.). The samples were kept in the Institute of Forestry, Belgrade (Serbia).

**Extraction and isolation**. Essential oils were obtained by simultaneous distillation and extraction from the needles (without twigs) with dichloromethane using the Likens-Nickerson apparatus (Liao *et al.* 2020). Each species was processed in four repetitions (trees).

**GC-FID and GC/MS analyses.** GC-FID and GC/MS analyses were carried out with an Agilent 7890A apparatus equipped with a 5975C MSD, FID, and an HP-5MSI fused-silica cap. col. (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed linearly rising from 60 to 315°C for 15 min; injector: 250°C; FID detector: 300°C; carrier gas, He (1.0 mL/min at 210°C), injection vol. 1 μL split ratio, 10:1. EI-MS (70 eV), *m/z* range 40–550.

**Compound identification.** The identification of all the compounds in the essential oils was matched by a comparison of their linear retention indices (relative to $C_8$–$C_{36}$ *n*-alkanes on the HP-5MSI column) and MS spectra with those of authentic NIST11 standards and home-made MS library databases.

**Statistical analyses.** The calculation of the arithmetic means, principal-component analysis (PCA), canonical discriminant analysis (CDA) and cluster analysis (Nearest Neighbour Method, Squared Euclidean Distance) were performed using Statgraphics Plus software (version 5.0; Statistical Graphics Corporation, USA).

## RESULTS AND DISCUSSION

The results of the terpene composition are presented in Table 1. Most of the analysed pines were richer in monoterpenes and sesquiterpenes than in diterpenes and triterpenes. In the Diploxylon pines of sect. *Pinus* subsect. *Pinus*, monoterpenes dominated over sesquiterpenes, i.e. monoterpene hydrocarbons dominated over sesquiterpenes. In subsect. *Pinaster* of the same section, sesquiterpenes dominated over monoterpenes (except in *P. pinea*) (Table 1; Fig. 1). Furthermore, two of the analysed pines from this subsection had a significant diterpene content (*P. halepensis* and *P. pinaster*), especially those which were oxygenated (Supplementary Table 1). One species from subg. Diploxylon (*P. ponderosa*) and all three of the analysed species of subg. Haploxylon (*P. peuce, P. strobus* and *P. wallichiana*) had abundant monoterpenes (Table 1; Fig. 1). This was particularly pronounced in *P. ponderosa* and *P. wallichiana*, which were the richest in monoterpene hydrocarbons (over 80%) (Table 1). Traces of triterpenes (squalene) were found in only two species (*P. nigra* subsp. *dalmatica* and *P. wallichiana*) (Table 1).

The profile of the main terpene compounds of *P. mugo* was as follows: $\alpha$-pinene >> $\delta$-3-carene = $\beta$-pinene > *trans*-caryophyllene > bornyl acetate (Supplementary Table 1), where 0.1–1.0% (=); 1.1 –5.0% (>); 5.1 –15.0% (>>); more than 15.1% (>>>) after Petrakis *et al.* (2001). In terms of dominant terpene compounds our sample is similar to Greek (Tsitsimpikou *et al.* 2001; Ioannou *et al.* 2014) and Serbian (Kosovo) (Mt. Šara) populations

**Table 1**. Terpene classes of 12 *Pinus* taxa.

| | Subgenus *Pinus* (Diploxylon) | | | | | | | | | Subgenus *Strobus* (Haploxylon) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Section *Pinus*, Subsection *Pinus* | | | | Section *Pinus*, Subsection *Pinaster* | | | | Section *Trifoliae*, Subs. *Ponderosae* | Section *Quinquefoliae*, Subsection *Strobus* | | |
| **Terpene classes** | *P. mugo* | *P. nigra* | *P. nigra* subsp. *dalmatica* | *P. sylvestris* | *P. halepensis* | *P. heldreichii* | *P. pinaster* | *P. pinea* | *P. ponderosa* | *P. peuce* | *P. strobus* | *P. wallichiana* |
| Monoterpene hydrocarbons | 58.3 | 66.2 | 50.3 | 52.2 | 16.2 | 30.0 | 16.7 | 49.9 | 78.3 | 67.7 | 58.2 | 81.6 |
| Monoterpenes oxygenated | 7.6 | 2.0 | 6.4 | 0.7 | 0.6 | 0.7 | 0.3 | 2.1 | 5.1 | 5.3 | 0.2 | 0.4 |
| **Total monoterpenes** | **65.9** | **68.2** | **55.7** | **52.9** | **16.8** | **30.7** | **17.0** | **51.0** | **83.4** | **73.0** | **58.2** | **82.0** |
| Sesquiterpene hydrocarbons | 20.6 | 27.4 | 29.5 | 32.3 | 33.5 | 64.0 | 17.8 | 35.9 | 12.3 | 16.0 | 20.9 | 11.3 |
| Sesquiterpenes oxygenated | 1.5 | 0.2 | 0.0 | 8.2 | 0.6 | 0.7 | 0.0 | 0.8 | 1.0 | 3.3 | 8.4 | 2.7 |
| **Total sesquiterpenes** | **22.1** | **27.6** | **29.5** | **40.5** | **35.1** | **64.7** | **17.8** | **36.7** | **12.3** | **19.3** | **31.3** | **14.0** |
| Diterpene hydrocarbons | 0.0 | 0.0 | 0.0 | 0.0 | 7.3 | 0.0 | 5.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| Diterpenes oxygenated | 1.1 | 1.9 | 1.3 | 0.0 | 21.8 | 0.0 | 39.2 | 4.2 | 0.0 | 0.4 | 0.4 | 0.5 |
| **Total diterpenes** | **1.1** | **1.9** | **1.3** | **0.0** | **29.1** | **0.0** | **44.4** | **4.4** | **0.0** | **0.4** | **0.4** | **0.5** |
| Triterpene hydrocarbons | 0.0 | 0.0 | tr | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | tr |
| **Total triterpenes** | **0.0** | **0.0** | **tr** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **tr** |
| **Aliphatic aldehydes and alcohols** | **1.7** | **0.0** | **0.3** | **0.0** | **0.0** | **3.8** | **0.0** | **0.0** | **0.0** | **0.2** | **0.8** | **0.0** |
| **Others** | 0.0 | 0.0 | 1.1 | 0.0 | 5.4 | 0.0 | 0.0 | 3.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| **n.i.** | 8.4 | 1.0 | 8.7 | 5.8 | 12.1 | 0.3 | 19.3 | 3.7 | 1.5 | 5.9 | 8.7 | 2.6 |
| **Traces** | 0.8 | 1.3 | 3.4 | 0.8 | 1.5 | 0.5 | 1.5 | 0.4 | 2.8 | 1.2 | 0.6 | 0.9 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |

n.i. - non identified compounds;
Others – aliphatic aldehydes and alcohols, aromatic acid esters, alkanes, bicyclic compounds, ethers, etc.

(Hajdari *et al.* 2015). However, in some other samples from the same mountain (Stevanovic *et al.* 2005; Basholli-Salihu *et al.* 2017; Mitić *et al.* 2017) and from the Julian Alps (Slovenia) (Bojović *et al.* 2016), $\delta$-3-carene is the dominant compound. In some previous papers, where other stationary columns were used, limonene, camphene, myrcene or $\delta$-3-carene and *p*-cymene were also noticeable (Kiliç & Koçak 2014; Bonikowski *et al.* 2015). It is important to underline that we identified five new compounds for *P. mugo*, but only in traces (these results are not presented in Supplementary Table 1).

The main compound in *P. nigra* subsp. *nigra* was $\alpha$-pinene, followed by $\beta$-pinene, germacrene D and *trans*-caryophyllene (Supplementary Table 1), which is consistent with previous findings (Chalchat & Gorunović 1995a; Roussis *et al.* 1995; Macchioni *et al.* 2003; Burzo *et al.* 2004; Šarac *et al.* 2013; Koutsaviti *et al.* 2015). A similar terpene profile was found using other capillary columns (Sezik *et al.* 2010), i.e. $\alpha$-pinene, germacrene D and caryophyllene dominated (Bojović *et al.* 2005).

The terpene profile of *P. nigra* subsp. *dalmatica* was also dominated by $\alpha$-pinene, similar to earlier investigations (Chalchat & Gorunović 1995b; Politeo *et al.* 2001). The profile of the sample investigated in our study is characteristic because *trans*-caryophyllene was the second ter-

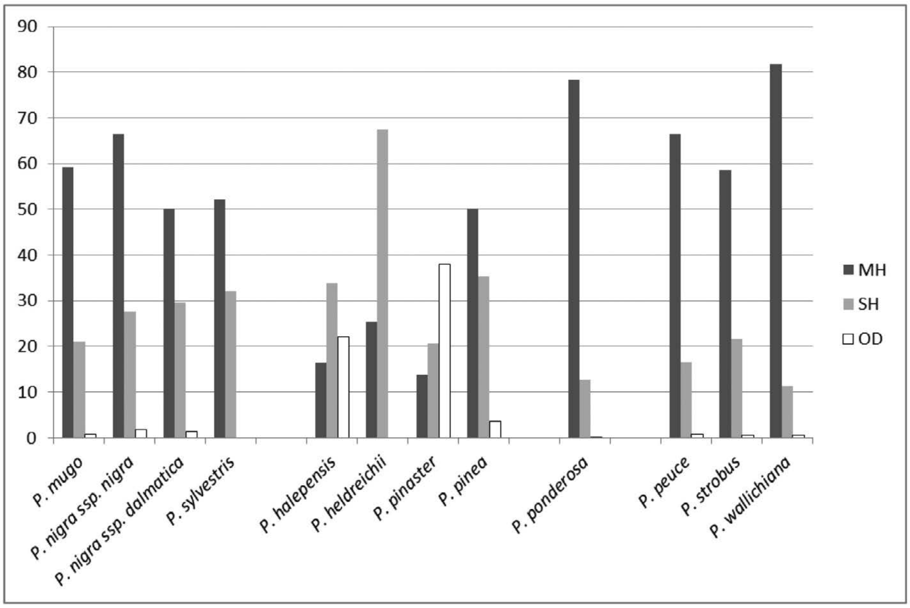


**Fig. 1**. The most abundant terpene classes of twelve *Pinus* taxa. MH – monoterpene hydrocarbons; SH – sesquiterpene hydrocarbons; OD – oxygenated diterpenes.

**Table 2**. Standardized coefficients for the first two canonical axes (CDA) of variation in 10 essential oil compounds from the discriminant functional analysis of 12 *a priori* groups. Significant coefficients are marked in bold.

| Compound | CA1 | CA2 |
|---|---|---|
| *a*-Pinene | 0.2218 | 0.32082 |
| ***b*-Pinene** | 0.0995 | **1.17695** |
| *d*-3-Carene | 0.0676 | 0.19949 |
| **Limonene** | 0.1282 | **1.00994** |
| ***trans*-Caryoph.** | 0.1011 | **0.92269** |
| **Germacrene D** | -0.3077 | **1.05179** |
| *d*-Cadinene | 0.3185 | -0.19399 |
| MS 172 | -0.4174 | -0.15951 |
| **Manool** | 0.0763 | **0.61470** |
| **Abietadiene** | **-0.8551** | 0.30742 |
| Eigenval | 159.5717 | 40.88967 |
| Cum.Prop | 0.6316 | 0.79346 |

pene compound, although in some previous papers it was borneol (Chalchat & Gorunović 1995b) or $\beta$-pinene (Politeo *et al.* 2001). Twenty five new compounds of *P. nigra* subsp. *dalmatica* were detected and identified in our results: phenyl ethyl 3-methyl butanoate (1.1%, No 26 in Supplementary Table 1) and twenty four compounds in traces (not presented).

The main terpene compounds in *P. sylvestris* were $\alpha$-pinene, $\beta$-cadinene and $\gamma$-cadinene (Supplementary Table 1). When other stationary columns were used, some other compounds in addition to $\alpha$-pinene and $\delta$-3-carene were also found in abundance (Idžojtić & Pfeifhofer 2001; Ustun *et al.* 2006, 2012).

$\alpha$-Pinene was the most abundant compound in all the listed pines of subsect. *Pinus*. The profiles of *P. nigra* subsp. *nigra* and *P. nigra* subsp. *dalmatica* were the most similar, possessing the same main compounds, with some slight differences – subsp. *nigra* had more $\beta$-pinene, while *P. nigra* subsp. *dalmatica* had more *trans*-caryophyllene. *P. mugo* had two more compounds in its main profile: $\delta$-3-carene and bornyl acetate. *P. sylvestris* was the most distant, with abundant $\delta$ and $\gamma$-cadinene. According to the nuclear EST-microsatellites, *P. nigra* subsp. *nigra* and *P. nigra* subsp. *dalmatica* were also closely related, but distant from *P. mugo* and *P. sylvestris* (Nikolić *et al.* 2018).

The *P. halepensis* terpene profile was characterized by *trans*-caryophyllene as the main constituent, which is similar to those from Greece (Ioannou *et al.* 2014; Koutsaviti *et al.* 2015), and Libya (Mohareb *et al.* 2008), but their terpene profiles differ in the second compound: manool (our results), and $\beta$-pinene, myrcene and No. 38 (Supplementary Table 1). However, in the needles of the Aleppo pine the most dominant terpenes are $\alpha$-pinene (Roussis *et al.* 1995; Macchioni *et al.* 2003), longifolene (Amri *et al.* 2013), or myrcene (Djerrad *et al.* 2016). In this paper, phenyl ethyl 3-methyl butanoate (4.9%, No. 26 in Supplementary Table 1), manool (20.8%, No. 42) and fifteen compounds found in traces (not presented) were detected for the first time.

*Pinus heldreichii* was unique in its abundant limonene and other sesquiterpenoides (Tables 1 & 2). Germacrene D was also the leading terpene compound in our previous investigations (Nikolić *et al.* 2015). The main terpene was limonene (Petrakis *et al.* 2001; Nikolić *et al.* 2007; Bojović *et al.* 2011; Ioannou *et al.* 2014;

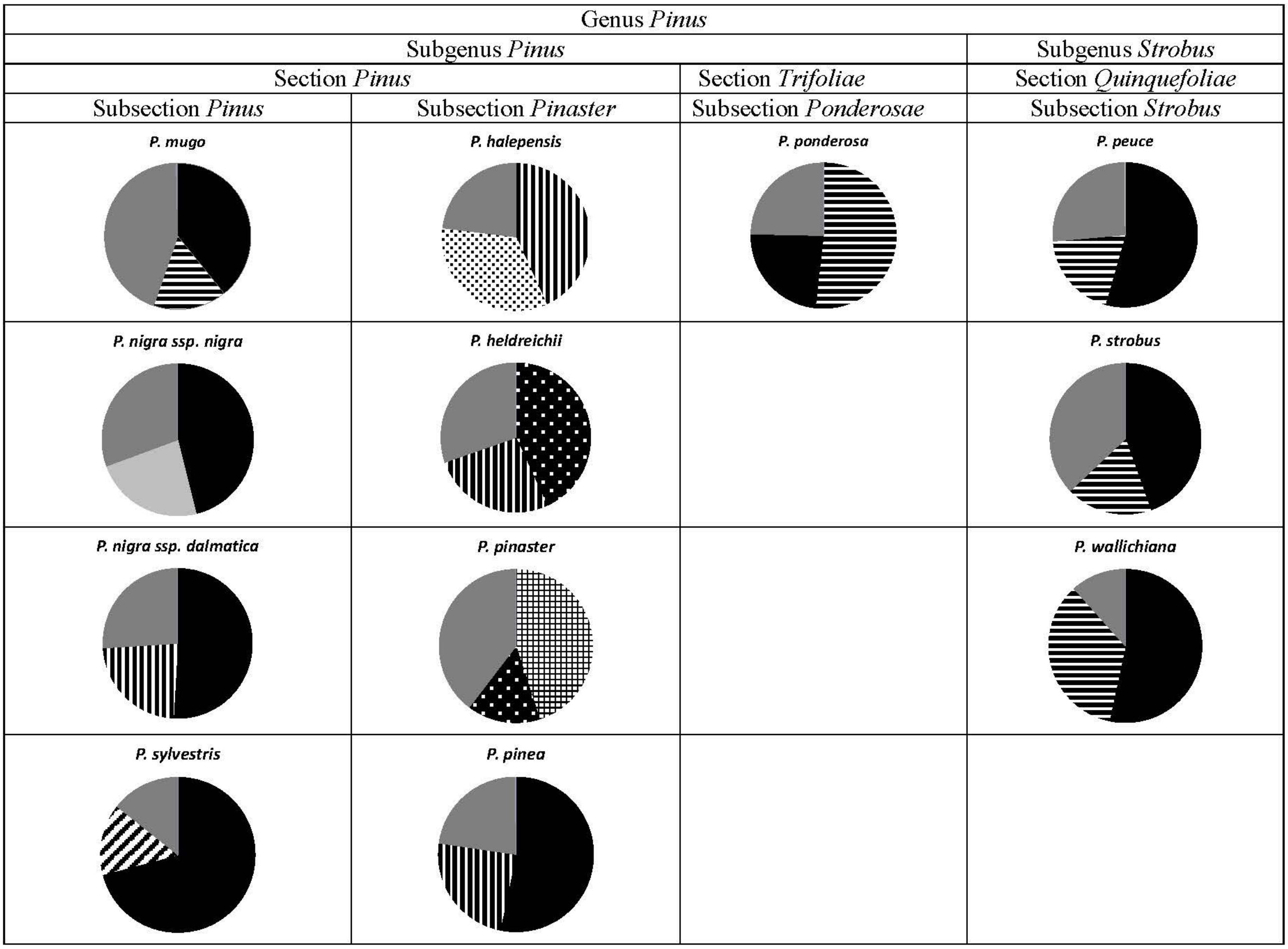


**Fig. 2**. The main terpene compounds of twelve *Pinus* taxa: *a*-pinene, *d*-3-carene, *b*-pinene, *(E)* – caryophyllene, germacrene D, d-cadinene, manool, abietadiene, and other compounds.

Basholli-Salihu *et al.* 2017). In our study, three new compounds were recorded for this species (in traces, the results are not presented).

The main terpene of *P. pinaster* was abietadiene, followed by α-pinene, *trans*-caryophyllene, germacrene and others. According to the dominant compound, our samples were most similar to the Greek populations (Koutsaviti *et al.* 2015), where isoabienol and sclarene sometimes dominated (Ioannou *et al.* 2014). In some earlier papers, *trans*-β-caryophyllene (Mimoune *et al.* 2013), isoabienol (Ioannou *et al.* 2014), or equal amounts of α-pinene and germacrene D (Koutsaviti *et al.* 2015) were the most abundant. Five new terpene compounds of *P. pinaster* were found only in this research, in traces (not presented).

In most of the pines, including *P. pinea*, α-pinene was the most dominant compound. Limonene dominates in literature data (Roussis *et al.* 1995; Macchioni *et al.* 2003; Ioannou *et al.* 2014). Limonene also dominated even if other stationary columns were used (de Simón *et al.* 2001; Amri *et al.* 2012; Demirci *et al.* 2015).

The four investigated species of subsect. *Pinaster* were clearly distinguished in terms of the dominant compounds: *trans*-caryophyllene, germacrene D, abietadiene and α-pinene (in *P. halepensis*, *P. heldreichii*, *P. pinaster* and *P. pinea*, resp.) (Supplementary Table 1). They also differed with respect to terpene classes and other main compounds (Fig. 2). According to the main terpene classes, as well as the main terpene compounds, *P. pinea* was more similar to *P. nigra* subsp. *nigra* and *P. nigra* subsp. *dalmatica* (Fig. 1). On the other hand, *P. halepensis* and *P. pinaster* were similar in terms of the significant level of diterpenes (Table 1).

Among all the investigated species of the subgenus *Pinus*, *P. ponderosa* had the highest values of monoterpene and the lowest percentage of sesquiterpene hydrocarbons (Table 1; Fig. 1). β-pinene was the dominant terpene, similar to samples collected from different countries (Adams & Edmunds 1989; Krauze-Baranowska *et al.* 2002; Burzo *et al.* 2004; Kelkar *et al.* 2006; Ioannou *et al.* 2014). Compared with previously obtained needle terpene profiles (Krauze-Baranowska *et al.* 2002; Kelkar *et al.* 2006), our sample had smaller amounts of

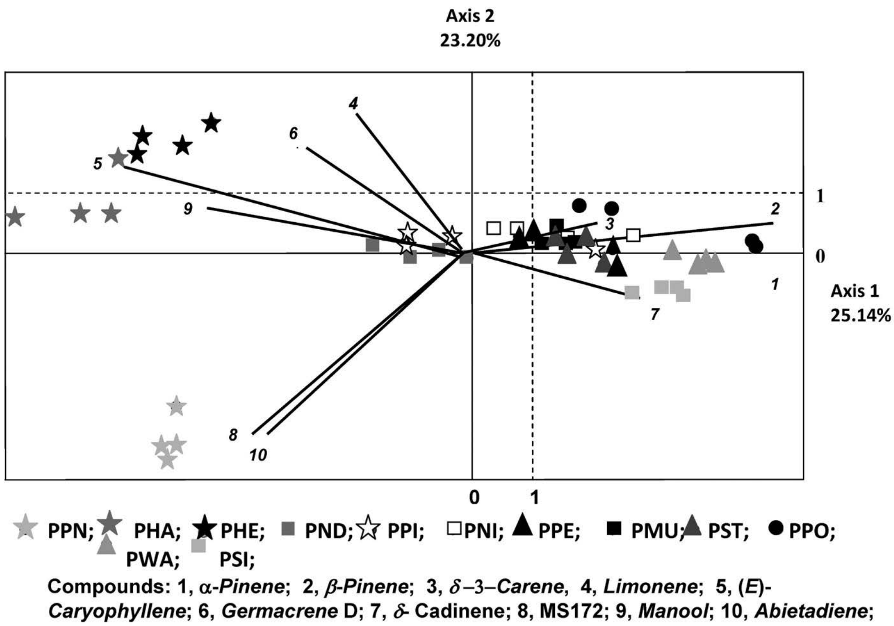


**Fig. 3**. Principle-component analysis (PCA) of ten selected terpenes isolated from 48 pine-tree samples of twelve *Pinus* taxa. The abbrevations for the *Pinus* taxa are given in the Material and Methods section.

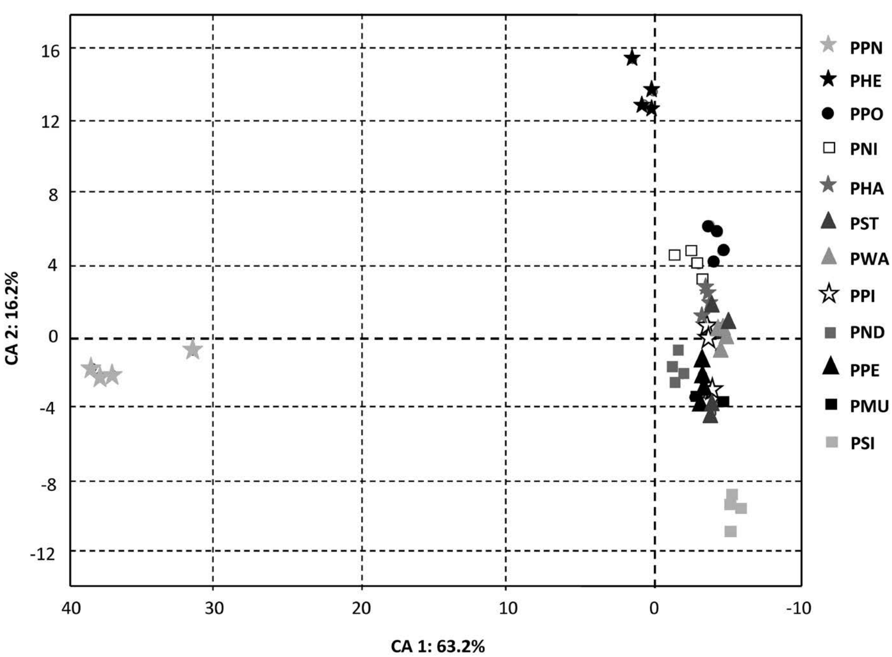


**Fig. 4**. Canonical discriminant analysis (CDA) of ten selected essential oil compounds isolated from 48 pine-tree samples of twelve *Pinus* taxa. For details of the abbreviations see the Material and Methods section.

estragole (methyl chavicol). Using other stationary columns, estragole emerged as one of the main compounds (Kurose *et al.* 2007). According to our findings and investigation, ca. 7 new compounds were found in *P. ponderosa*: α-pinene (19.8%, No. 4), guiaol (1.1%, No. 33) (Supplementary Table 1), and five compounds in traces (not presented).

The main terpene compound in *P. peuce* was α-pinene, which is consistent with our previous investigation (Nikolić *et al.* 2008). Comparing our findings with the results in the literature, we found eight additional compounds in *P. peuce*, methyl chavicol (1.4%, No. 16, Supplementary Table 1) and seven compounds in traces (not presented).

α-Pinene and β-pinene were the main terpene compounds in *P. strobus*, as in some earlier findings (Koutsaviti *et al.* 2015). When a different stationary column was used, α-pinene and β-myrcene strongly dominated (Kiliç & Koçak 2014). In our study, we

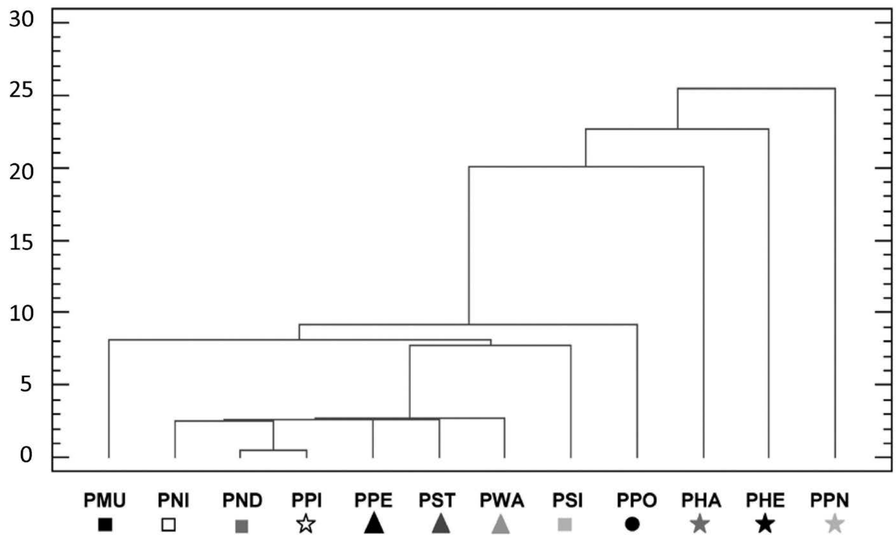


**Fig. 5**. Dendrogram based on the nearest neighbour method (squared Euclidean distance) of the studied species. For details of the abbreviations see the Material and Methods section.

found 14 additional compounds in *P. strobus*, all in traces (these results are not presented).

The *P. wallichiana* terpene profile: α-pinene >>> β-pinene >>> (*E*)-caryophyllene was similar to some literature data (Dar *et al.* 2012; Ioannou *et al.* 2014; Dambolena *et al.* 2016) where β-pinene was the most abundant. In the present study, 9 additional compounds of *P. wallichiana* were recorded, all in traces (these results are not presented).

All three of the analysed species of sect. *Quinquefoliae* had high amounts of α-pinene. The profile of *P. wallichiana* was distant from *P. peuce* and *P. strobus*. Nuclear DNA analysis also showed this distance (Nikolić *et al.* 2018).

According to the PCA data analysis (Fig. 3) high values of *trans*-caryophyllene and manool, as well as MS172 and abietadiene, contributed to the greatest separation of *P. pinaster*, *P. halepensis* and *P. heldreichii* (all from subsect. *Pinaster*).

Based on the CDA data analysis (Fig. 4), *P. pinaster*, *P. heldreichii* and *P. sylvestris* were the most distant. Additionally, Root 2 distinguished two groups: 1. *P. nigra* subsp. *dalmatica*, *P. peuce*, *P. strobus* and *P. mugo* and 2. *P. ponderosa*, *P. nigra* subsp. *nigra*, *P. halepensis*, *P. pinea* and *P. wallichiana*. The distribution of abietadiene has the greatest influence on Root 1, while Root 2 was mainly determined by the content of β-pinene, limonene, germacrene D, *trans*-caryophyllene and manool (Table 2).

The eight species with the most abundant α-pinene were the most similar in the cluster analysis and mainly belonged to subsect. *Pinus* and *Strobus* (Fig. 5). According to the cluster analysis of *n*-alkanes, sect. *Pinus* and *Pinaster* diverged from the sect. *Strobi* (Nikolić *et al.* 2020). In previous RAPD analysis, *P. heldreichii* (sect. *Pinaster*) was also distant from *P. nigra* and *P. sylvestris* (sect. *Pinus*) (Kovačević *et al.* 2013). The separation of the pines of subsect. *Pinaster* was also confirmed by nuclear DNA analyses (Nikolić *et al.* 2018). Wang & Wang (2014) examined sequence variation in nine mtDNA regions in 36 pine species representing nine of the 11 subsections of the genus. According to these authors, the mtDNA phylogeny exhibits more geographical coherence than genealogical relationships between lineages, and generates more paraphyletic groups than cp and nuclear DNA trees. More recently, Zeb *et al.* (2019) analysed the sequences of 97 *Pinus* plastid genomes, including four newly sequenced genomes and 93 previously published plastomes, to explore the evolution and phylogenetic relationships in the genus *Pinus*. The phylogenetic tree (Zeb *et al.* 2019) based on 60 coding plastid genes showed that the *Pinus* species could be divided into two diverged clades comprising the subgenera *Strobus* (single needle section) and *Pinus* (double needles section). The grouping of the species analysed in our research, based on terpene profiles, is consistent with the generic, sectional and subsectional position provided by Zeb *et al.* (2019).

## CONCLUSIONS

Our results showed that in the main terpene profile, α-pinene was the most abundant compound in eight pines. In the PCA analysis of the terpenes of twelve *Pinus* taxa, the greatest separation was in the cases of *P. pinaster*, *P. halepensis* and *P. heldreichii* (all from subsect. *Pinaster,* sect. *Pinus*). In CDA, the most distant were *P. pinaster*, *P. heldreichii* (subsect. *Pinaster*) and *P. sylvestris* (subsect. *Pinus)*. Despite the fact that a very limited number of pine species was analysed in this research, there are some indications that terpene profiles might be useful in the differentiation of infrageneric taxa of the genus *Pinus*. Further sampling and investigation of other species belonging to different subgenera are required in order to gain a better picture of the possiblee delimitation of subgenera, sections and subsections based on terpenes. Before drawing any conclusions, caution should be applied when comparing terpene diversity results when different stationary columns are used. Our results of the terpene cluster analysis in the pines are consistent with the phylogenetic analyses based on the cpDNA.

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REZIME

Botanica SERBICA

## Terpenski odnosi između nekih mekih i tvrdih borova

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U ovom proučavanju objavili smo hemijski sastav etarskih ulja dobijenih iz iglica 12 taksona roda *Pinus*, koji pripadaju podrodovima *Pinus* (sekcije *Pinus* i *Trifoliae*) i *Strobus* (sekcija *Quinquefoliae*). U većini istraživanih taksona monoterpeni dominiraju nad seskviterpenima kod oba podroda, osim kod uzoraka podsekcije *Pinaster* podroda *Pinus*. *α*-Pinen je najobilniji kod 8 borova, ali kod nekih taksona dominantni su *trans*-kariofilen, germakren D, abietadien i *β*-pinen. U subcekciji *Pinaster* dominantne komponente variraju od vrste do vrste. Nadalje, *P. halepensis* i *P. pinaster* imaju najveći procenat diterpena, dok je *P. heldreichii* najbogatiji u germakrenu D. U PCA i klaster analizama pokazale su se najudaljenije tri vrste subsekcije *Pinaster*: *P. halepensis*, *P. pinaster* i *P. heldreichii.* Nadalje, u analiziranim vrstama roda *Pinus* diskutovane su moguće taksonomske implikacije terpenskog profila.

**Ključne reči:** *Pinus*, diverzitet, analiza glavnih komponenti, kanonijska diskriminantna analiza, klaster analiza