



Variability of volatiles in *Pinus cembra* L. within and between trees from a stand in the Salzburg Alps (Austria) as assessed by essential oil and SPME analysis

Remigius Chizzola · Katharina Müllner

Received: 30 January 2020 / Accepted: 30 August 2020 / Published online: 16 September 2020
© The Author(s) 2020

Abstract *Pinus cembra* L. is a coniferous European mountain range tree rich in oleoresins and essential oils. Twig tips with needles are the most common tree parts used to obtain essential oils. As the whole tree contains volatiles, the essential oil composition from different parts was studied, including twig tips with needles from the bottom, the top and the cone bearing branches, cones, twigs without needles from the lower and upper part of the crown as well as wood and bark from the trunk. The variability in essential oil composition between these plant parts and between individual trees has been studied using multivariate statistical analyses. α -Pinene was present in all samples, being highest in cones (49.3%) and lowest in sapwood (0.7%). Twig tips with needles from different parts of the crown had similar essential oil

composition with α -pinene (43.9–48.3%), β -phellandrene (13.1–17.2%), β -pinene (6.6–9.3%), germa-crene D (5.1–6.8%) and limonene (4.1–6.1%) as main compounds. Twig essential oils had usually more β -phellandrene (23.9–29.8%) than α -pinene (23.3–24.3%) and also appreciable amounts of β -pinene (13.5–15.1%) and limonene (11.9–17.9%). Cone essential oils contained mainly α -pinene, β -pinene (20.1%) and limonene (13.9%). The essential oils from wood and bark were rich in diterpenes as cembratrienol (4.9–21.4%), cembrene (4.8–14.3%) and methyl daniellate (2.7–6.8%). Sapwood distillates contained also notable amounts of alkane derivatives. Finally, the solvent free SPME technique has been employed to analyse the volatiles in the plant parts. For needles and cones the observed patterns were in good accordance with the compositions of the respective essential oils.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10722-020-01006-2>) contains supplementary material, which is available to authorized users.

R. Chizzola (✉)
Institute of Animal Nutrition and Functional Plant
Compounds, University of Veterinary Medicine Vienna,
Veterinärplatz 1, 1210 Vienna, Austria
e-mail: Remigius.Chizzola@vetmeduni.ac.at

K. Müllner
Competence Center for Wood Composites and Wood
Chemistry – Wood K plus, Altenberger Straße 69,
4040 Linz, Austria
e-mail: katharina.muellner@gmail.com

Keywords Pinaceae · *Pinus cembra* · Essential oil · α -Pinene · Cembrene · Intraindividual variation

Introduction

Pinus cembra L. (Pinaceae), also known as swiss stone pine, cembran pine, ceder pine, or arrola pine (Apetrei et al. 2011), naturally occurs in two disjunct regions: in the European Alpine chain (Austria, Switzerland,

Italy, France) from 1500 to 2500 m a.s.l., and the Carpathian Mountains (Poland, Slovakia, Ukraine, Romania) from 1300 to 1700 m a.s.l. (Wieser et al. 2006; Wojnicka-Półtorak et al. 2015). Isolated trees can be found up to 2850 m a.s.l. in the Alps and up to 1985 m a.s.l. in the Carpathian Mountains (Wojnicka-Półtorak et al. 2015). *Pinus cembra* occurs in pure or mixed stands with *Picea abies* L. Karst, *Larix decidua* Miller, *Pinus sylvestris* L. and *Pinus mugo* Turra, and often reaches the tree-line. Natural competition with associated tree species as well as human activities, like shepherding, alpine farming and timber extraction strongly affects the distribution of *P. cembra* (Boden et al. 2009; Zong et al. 2010) and has led to displacement to marginal habitats (Höhn et al. 2009).

P. cembra is a five-needled evergreen conifer tree (Boden et al. 2009), growing up to 25 m in height and 1.7 m in trunk diameter (Schütt 2014). Synzoochory dispersion of the wingless seeds is mostly done by the European nutcracker (*Nucifraga caryocatactes* L.). The bird caches seeds for later consumption in winter and, if failing to recover, the seeds can germinate. *P. cembra* trees are well adapted to subalpine climate conditions (Boden et al. 2009; Caudullo and de Rigo 2016; Zong et al. 2010) and are highly resistant to abiotic stress factors at high altitudes (Apetrei et al. 2013). They are frost-hardy and can persist winter temperatures down to $-43\text{ }^{\circ}\text{C}$ (Caudullo and de Rigo 2016; Schütt 2014). *P. cembra* is supposed to be reaching the age of 1000 years (Schütt 2014; Schweingruber and Wirth 2009).

Regionally *P. cembra* gained economic significance. It is listed as economically important plant (Smith 2017), in alpine northern Italy seeds are sold as food (Ducci et al. 2014). In the Austria federal land Tyrol, *P. cembra* accounts for about 2.3% of the usable trees in forestry (Landwirtschaftskammer Tirol 2014) and the price for its wood showed considerable fluctuations in the last years (Land 2018). So, in alpine regions, *P. cembra* wood is used for construction and furniture industry while the essential oil has various applications in medicine and aromatherapy, in local liquor production and as a fragrance in cosmetic (Thaler-Rizzoli and Gasteiger 2017). Furthermore, the essential oil is said to have positive effects on indoor climate although scientific evidence is lacking. Recently, efforts have been undertaken to support rural regions in promoting uses of *P. cembra*. The interest in non-timber products of this tree is mainly

based on aromatic properties of its parts (Weiss et al. 2017).

Several reports mention α -pinene, limonene, β -phellandrene and β -pinene amongst the main essential oil compounds, with concurrently low amounts of sesquiterpenes (Apetrei et al. 2013; Kubeczka and Schultze 1987; Lis et al. 2017; Nikolic et al. 2018). Essential oils from woody parts and cones additionally contain some diterpenes (Lis et al. 2017). Volatiles emitted by *P. cembra* cones of different geographic origin include α -pinene (67–70%), β -pinene (18–20%) and limonene/ β -phellandrene (8–11%) (Dormont et al. 1998). A commercial *P. cembra* needle essential oil from Italy contained 22% (+)- α -pinene, 12% (-)- α -pinene, 0.1% (+)- β -pinene, 12% (-)- β -pinene, 33% (-)-limonene and no (+)-limonene (Ochocka et al. 2002).

Analysis of essential oil composition has been focused only on a few plant parts. Yet, other parts could also contain substantial amounts of essential oils and natural aromes. Therefore, we focussed our study (1) to investigate the variability in essential oil composition from different parts of *P. cembra*, (2) to obtain information to which extent trees from a stand differ in essential oil composition, and (3) to investigate the pattern of volatiles obtained with the solvent free SPME technique as it is close to the fraction perceived by the nose and can be considered as responsible for the fragrance. A comparison to distilled oils is attempted.

The findings may support further exploitation of *P. cembra* as a source of essential oils and natural aromes.

Materials and methods

Plant material

Samples from seven individual *P. cembra* trees were collected in September 2015 in Austria (Salzburg, Thomatal, N $47^{\circ}02' 35.8''$, E $13^{\circ} 45' 26.6''$) at an altitude of 2003 m. The age of the individuals ranged from 120 to 175 years as estimated by counting the annual growth rings. Due to the very slow growth of *P. cembra* an uncertainty of ± 10 years was not avoidable. The distance between the sampled individuals ranged from 5 m (tree N^o. 1 to tree N^o. 2) to 700 m (tree N^o. 1 to tree N^o. 7). A voucher specimen was

deposited in the Herbarium of the University of Vienna (WU Generale, <http://herbarium.univie.ac.at/>).

From each individual tree the following parts were collected and denoted as follows: shoots with needles (NT) and twigs without needles (max. 2 cm in diameter) (TT) from treetop, shoots with needles (NB) and twigs without needles (TB) from the lower third of the tree, shoot with needles from cone bearing twigs (NC), one year-old cones from treetop (CO), bark from the trunk (BA), heartwood (HW) and sapwood (SW) from the lower part of the trunk. Heart- and sapwood were cut in pieces of 2–5 cm. Only healthy appearing plant parts were taken. The plant parts were dried in the ambient air and kept at room temperature until analysis.

Essential oils extraction by hydrodistillation

The materials subjected to distillation were about 10 g for CO, NB, NC, NT and TT, 20 g for TB and 50 g for BA, HW and SW. These plant materials were further chopped and hydrodistilled with 400 mL of double distilled water in a Clevenger type apparatus for 3 h. The amount of essential oil obtained was read from the capillary of the distillation unit. The collected volatile fractions were stored at $-18\text{ }^{\circ}\text{C}$ until further GC and GC-MS analyses. Prior to analysis, 5 μL of the essential oil samples were diluted with 495 μL hexane containing hexadecane (0.107 mg/mL) as internal standard.

GC/MS and GC/FID

The analyses were carried out on an Agilent Technologies 7890A gas chromatograph equipped with a 5975 C quadrupole mass selective detector, a flame ionization detector (FID) and a CTC-PAL autosampler (Agilent Technologies, Santa Clara, CA, USA) as described in Chizzola et al. (2018) with slight modifications. The separation was done on a 30 m \times 0.25 mm fused silica column coated with 0.25 μm HP5-MS. The compounds eluting from the column were distributed with a Deans switch at equal proportions to the detector of the mass spectrometer (MSD) and FID. The temperature program of the oven was: isothermal at 50 $^{\circ}\text{C}$ for 1 min, then increasing to 220 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}/\text{min}$ and increasing further to

280 $^{\circ}\text{C}$ at a rate of 15 $^{\circ}\text{C}/\text{min}$. The split of the injector was set at 1:20. The injection volume was 1 μL . The total ion current (m/z 40 to 500) from the MSD was used to identify the compounds according their mass spectra and their retention indices (Adams 2007; McLafferty 1989). As the analytical column did not separate limonene and β -phellandrene well, these two compounds were deconvoluted using the AMDIS-software within the NIST spectral library. The FID operated at 250 $^{\circ}\text{C}$ with 400 mL/min air flow and 40 mL/min hydrogen flow. The peak areas of the FID signal were used to calculate the percental composition of the oils without any correction.

SPME

From three individual trees the woody fractions (BA, HW, SW, TT) as well as the cones (CO) and needles only from the top twigs (NT-N) were also analysed by SPME. Small amounts of the samples (about 120 mg for HW and SW and about 30 mg for other parts) were finely chopped and put into a 10 mL vial together with a filter paper disc (6 mm in diameter) soaked with 10 μL of cyclododecanone as internal standard (0.4 mg/mL in methanol). The vial was tightly closed with a septum and further processed in the CTC-PAL autosampler with the mounting for SPME fibers. The SPME fiber (PDMS-DVB, polydimethylsiloxane-divinylbenzene, Supelco, Bellefonte, PA, USA) was exposed for 30 min at 50 $^{\circ}\text{C}$ while stirring to the headspace of the sample. Afterwards the fiber was introduced into the injection port of the GC system and desorbed for 5 min at 250 $^{\circ}\text{C}$ at a split ratio of 10:1. The conditions for GC/MS and FID were the same as described above. Each sample was analysed thrice with SPME.

Statistical analysis

The statistical analyses were done with the package SPSS for Windows, version 24.0 (IBM Corporation, Armonk, NY, USA). A hierarchical cluster analysis (HCA) using the squared Euclidian distance with linkage between groups was carried out to group the plant parts according to the composition of their essential oils. Furthermore, the complex interplay of major essential oil compounds was studied by principal component analysis (PCA) using the samples as cases and the essential oil compounds as listed in

Table 1 as variables. The data were Z-transformed by the software before calculating the loadings of the variables and scores of the samples. Canonical discriminant analysis (CDA) tested whether the sampled could be correctly assigned to the originating tree or plant part.

Results and discussion

Essential oil yield

The hydrodistillation of the various plant parts gave different oil yields as presented in Table 1. Essential oil yield was highest in the unripe cones (CO), reaching 3.0% v/w. Twig tips with needles, which are commonly used for commercial essential oil production, were collected from the upper (NT) and lower part (NB) of the trees as well as from cone bearing twigs (NC). In those parts oil yield ranged between 2.3 and 2.5% v/w. Twigs from the tree top (TT, 2.2% v/w) were richer in essential oils than those from the bottom (TB, 1.1% v/w) while bark (BA) and wood (HW, SW) had very low oil content. Varying essential oil contents were reported in this species: High oil content in unripe cones (2.4% v/w) was also reported from trees in an arboretum in Rogow, Poland. These plants had about 0.46–0.66% v/w oil in needles and twigs and 0.94% v/w in the bark while the wood was also low in volatiles (Lis et al. 2017). The higher oil content in the bark in comparison to the present results may arise from the circumstance that the authors analysed bark from branches. High oil content was also reported in needles (1.96% v/w) and twigs (2.45% v/w) from trees in Romania (Apetrei et al. 2013), while needles from trees grown near Trento in Italy gave 0.61% v/w essential oil (Domrachev et al. 2012).

Essential oil composition

To compare the essential oil compositions from the different plant parts, the similarities among the oils have been first studied with a hierarchical cluster analysis (Fig. 1). The highest similarities were found between the three twig tips with needles (NT, NB and NC). These samples contained α -pinene as major

compound, averaging 43.9–48.3% of the total compounds quantified (Table 1). Available literature reported α -pinene contents in oil from needles or twigs with needles ranging from 21.1% (Ioannou et al. 2014) to 69.1% (Apetrei et al. 2013). In the present essential oils, β -phellandrene, β -pinene, limonene and germacrene D were next major compounds in twig tips with needles. While most previous reports mention up to 20% germacrene D in needle oils (Ioannou et al. 2014), this compound occurred only in trace in oils from Romania (Apetrei et al. 2013).

Together with the cones (CO) and the twigs from the top (TT) and the bottom (TB) of the trees, twig tips with needles were grouped in a cluster clearly distinct from the cluster formed by bark (BA) and wood (HW, SW) samples. In cones, α -pinene was also found to be the main oil compound (49.3%) followed by β -pinene (20.1%) and limonene (13.9%). In twig oils (TT, TB), the relative amount of the components decreased in the order β -phellandrene, α -pinene, limonene, β -pinene. Contrary to the cones, twig and needle oils contained more β -phellandrene than limonene. This result is in some contrast to the finding of Lis et al. (2017) who reported more limonene than β -phellandrene in various parts (needles, twigs, cones). In sum in needles, monoterpenes prevailed in the essential oils of needles, cones and twigs.

Wood (HW, SW) and bark (BA) from the trunk were low in essential oils. The bark oil was rich in monoterpenes again with α -pinene at the top, followed by β -phellandrene, limonene and β -pinene, similarly as in needles. There were also low amounts of δ -3-carene and α -terpineol. However, the bark essential oil contained also sesquiterpenes as α -cadinol and α -bisabolol, and diterpenes. Diterpenes, finally, formed the main part of the wood oils (HW, SW). The main diterpenes identified were cembrene (= thunbergene), cembratrienol (= thunbergol) and methyl daniellate (= methyl lambertianate). To compare, the essential oil from *Pinus heldreichii* heartwood contained 23.8% cembrene, 5.6% cembrene A, 28.7% limonene and 6.4% α -pinene (Graikou et al. 2012). In our study, the main diterpene in cones was abieta-6,8(14)-dien-18-al (2.7%). Likewise, cone oils from plants grown in a Polish arboretum contained 5.7–10.0% of this compound (Lis et al. 2017) that occurred also in oils from *Pinus mugo* (Venditti et al. 2013). In contrast to all other plant parts, sap wood oil contained various alkane derivatives participating for about 30% of the

Table 1 Essential oil yield and composition (%) obtained from the different parts of *Pinus cembra*

Compound	RI	NT		NB		NC		CO		TT		TB		BA		HW		SW	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oil yield (% v/w)		2.5	0.5	2.4	0.3	2.3	0.4	3.0	0.4	2.2	0.3	1.1	0.6	0.2	0.2	0.1	0.1	0.1	0.1
Monoterpenes																			
α -Pinene	942	44.5	5.8	48.3	6.3	43.9	5.4	49.3	17.5	23.3	3.2	24.3	2.2	25.2	16.3	17.9	22.0	0.7	0.7
Camphene	957	1.7	0.9	1.6	0.8	1.5	0.8	0.6	0.2	4.0	2.3	3.8	2.5	2.5	1.6	0.3	0.4		
β -Pinene	985	8.2	3.6	6.6	3.1	9.3	4.2	20.1	7.5	15.1	4.8	13.5	4.3	3.1	2.9	1.7	1.7		
Myrcene	993	0.9	0.2	0.8	0.2	0.7	0.2	3.5	6.7	1.6	0.2	1.5	0.1	0.2	0.2	0.0	0.1		
δ -3-Carene	1016	0.1	0.1	0.2	0.1	0.3	0.2			0.5	0.4	0.8	0.2	4.8	1.5	4.0	3.9	0.5	0.5
Limonene	1039	4.1	4.0	4.9	5.8	6.1	5.5	13.9	9.2	17.9	13.3	11.9	9.3	3.0	4.6	0.4	0.5		
β -Phellandrene	1039	17.2	6.9	15.8	7.9	13.1	6.1	2.9	2.6	23.9	10.2	29.8	9.5	4.8	4.4	0.5	0.5		
Borneol	1175	0.1	0.2	0.2	0.1					0.3	0.2			1.8	0.8	0.8	0.7	t	
α -Terpineol	1197	0.4	0.3	0.2	0.1	0.6	0.2	0.3	0.1	0.3	0.2	0.1	0.1	3.2	1.2	3.7	3.0	0.9	0.9
Bornyl acetate	1293	0.7	0.4	0.3	0.3	0.9	0.6	0.4	0.1	2.3	2.1	0.8	1.3	1.9	2.1	0.3	0.2	1.1	1.1
Sum		77.9		78.9		76.4		91.0		89.2		86.5		45.7		29.6		3.2	
Sesquiterpenes																			
Sibirene	1417									t		t		1.5	0.7	1.1	0.3	0.8	0.4
α -Amorphene	1490	0.8	0.3	0.7	0.2	0.2	0.3	t	0.1	0.1	0.1	0.1	0.1	1.5	0.8	1.4	0.5	1.0	0.4
Germaacrene D	1498	6.4	1.8	6.8	1.6	5.1	1.9	0.6	0.7	0.4	0.2	0.3	0.2	0.1	0.2	t		0.1	0.1
δ -Cadinene	1537	2.3	0.6	2.4	0.5	1.5	0.5	t		0.2	0.1	0.4	0.1	1.2	0.4	1.0	0.3	0.5	0.4
δ -Cadinol	1662	0.1	0.1	0.1	0.0	0.2	0.0			0.1	0.1	0.1	0.1	4.5	2.3	5.1	2.5	6.2	1.7
α -Bisabolol	1697	0.3	0.5	0.2	0.2	0.1	0.2	t		0.3	0.3	0.5	0.4	1.8	1.9	1.1	1.1	1.9	1.9
Sum		9.9		10.2		7.1		0.6		1.1		1.4		10.6		9.7		10.5	
Diterpenes																			
Cembrene	1955	0.2	0.0	0.1	0.0	0.4	0.1	0.1	0.1	0.7	0.5	0.5	0.2	4.8	1.7	14.3	6.1	5.0	2.4
Iso-Cembrene	1968	t		t		0.2	0.0	t		0.3	0.2	0.2	0.1	1.2	0.4	3.2	1.4	1.6	0.8
U242	2017					0.0	0.0	0.1		t		t		0.2	0.3	2.4	2.3	1.2	0.7
Cembraatrienol	2087	0.3	0.1	0.3	0.1	0.8	0.4	0.1	0.1	0.8	0.7	0.7	0.3	4.9	2.1	10.0	5.3	21.4	6.6
3,5-Methoxy-stilbene	2249							t								2.0	2.4	0.3	0.4
Abieta-6,8(14)-dien-18-al	2267	0.3	0.1	0.3	0.2	0.4	0.1	2.7	1.1	0.7	0.3	0.7	0.4	1.8	1.2	3.2	1.4	2.5	1.2
Methyl-daniellate	2350	0.9	0.4	1.2	0.4	0.6	0.2	0.2	0.0	1.4	0.5	2.3	1.9	6.8	6.5	2.7	1.5	3.8	2.2
Sum		1.5		1.9		2.4		3.1		3.9		4.4		19.7		37.8		35.8	

Table 1 continued

Compound	RI	NT		NB		NC		CO		TT		TB		BA		HW		SW		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Alkane derivatives																				
n-Hexanol	868																		< 0.05	
n-Heptanol	970																		0.8	1.0
Hexanoic acid	981																		1.1	1.3
n-Octanal	1003																		1.7	1.3
3E-Octen-2-one	1042																		1.7	1.0
2E-Octenal	1061																		3.2	1.0
2-Nonanone	1093																		0.3	0.4
n-Nonanal	1105																		5.0	2.2
2E-Nonenal	1163													0.1					0.6	0.3
Octanoic acid	1175																		2.7	2.8
n-Decanal	1207																		1.2	0.3
2E,4E-Nonadienal	1218																		0.2	0.2
2E-Decenal	1266																		2.9	1.0
Nonanoic acid	1275												0.1						5.2	3.7
2E,4Z-Decadienal	1297																		0.1	0.2
2E,4E-Decadienal	1321													0.3					0.5	0.3
2E-Undecenal	1368																		2.8	1.1
5-Undecen-4-one	1523																		0.3	0.3
Sum																			30.3	
Total Sum		89.5		90.8		86.0		94.7		94.4		92.6		81.2		77.3			49.5	

Mean and standard deviation (SD) from 7 trees, t: traces (< 0.05%)

Shoots and needles from the treetop (NT) the bottom (NB) and the cone bearing twigs (NC), one year old cones from treetop (CO), twigs without needles from the treetop (TT) and the bottom (TB), bark (BA), heartwood (HW) and sapwood (SW)

U242: unidentified compound, 242 g/mol, *m/z* (%): 151 (100), 242 (46), 91 (33), 152 (12), 65 (11), 77 (9), 243 (8), 78 (7), 121 (5), 165 (4)

Means are presented in ordinary fonts while standard deviations (SD) are given in italics

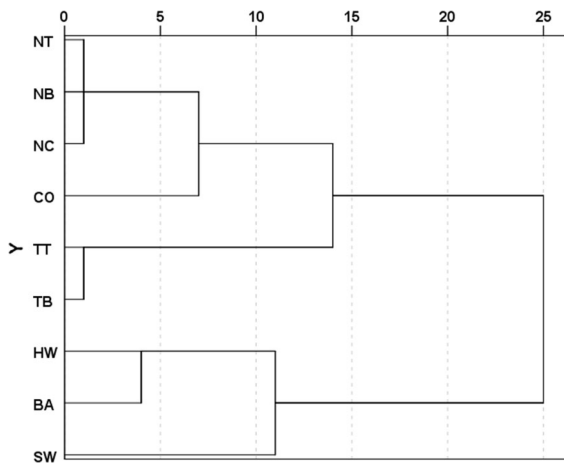


Fig. 1 Dendrogram showing the similarities between the plant parts of *Pinus cembra* according to their oil composition

essential oil content. The composition of this fraction is presented in Table 2 with nonanoic acid, nonanal and 2*E*-octenal as the dominant compounds. Lis et al. (2017) analysed oils from bark and wood from 2 to 3 years old branches and found that the wood oil contained about 16% diterpenes and the bark oil 1.4%, but no alkane derivatives were reported.

This differentiation between the parts of the trees has further been visualized calculating a principal component analysis (PCA). From 23 essential oil compounds as variables, the PCA calculated seven components having eigenvalues greater than one and representing together 86.1% of the total variance. The first axis accounted for 40.5% and the second for 12.1% of the variance. Figure 3 represents the scoring of the samples on the components. Wood and bark samples could well be separated from cones, twigs and twigs with needles by the first component where the latter scored with positive values (Fig. 2A). This differentiation is based mainly on monoterpenes with negative factor 1 loading occurring mainly in needles, twigs and cones while sesquiterpene and diterpene compounds showing positive factor 1 loadings were characteristic for the woody parts of the stem. In addition, bark and heartwood samples had the highest δ -3-carene contents, so this compound had a similar positive component 1 loading as sesquiterpenes and diterpenes. Similarly, bark and some heartwood samples are represented in the lower right quadrant on the factor 3 and 4 plottings (Fig. 3a, b). Twig tips

with needles (NC, NT, NB) had a negative component 2 score according to their high α -pinene content but also with their germacrene D and δ -3-cadinene levels as these compounds had a negative component 2 loading. Twig (TT, TB) and cone (CO) oils scored positively on factor 2 according to their relative content of β -phellandrene, β -pinene and limonene. Especially for wood and bark samples, the spread of the individual samples from a plant part on the plane of the factor scores shows that there is some diversity between the seven analysed trees. This diversity is also the cause of some high standard deviations in respect to the means in Table 1. In cone oils, α -pinene varied from 26.4 to 71.7%, in bark oils from 6.8 to 50.5%. Three samples from the heart wood had less than 0.5% α -pinene while in the remaining four samples this compound ranged from 14.4 to 60.3% (data with the supplementary material). Sapwood showed a considerable variability in alkane derivatives (Table 1) and the main diterpene cembratrienol (9.4–29.1%).

We used canonical discriminant analysis (CDA) for a further characterization of the essential oil variability. In this multivariate analysis, objects have to be assigned to predefined groups. In the present analysis, we tested to relate the essential oil samples to the plant parts (Fig. 4a) or to the individual trees (Fig. 4b). Sapwood essential oils had strong negative function 1 scores and were differentiated clearly from all other oils (Fig. 4a). Function 2 distinguished cone, twig and needle essential oils from heartwood and bark oils. The latter two were also distinguished. Concerning the differentiation of the individual trees, there were two outstanding trees, number 1 and 7, and some overlapping between other trees. Altogether, 54 of 62 samples could be correctly classified in CDA. Tree 1 had the highest limonene contents in all plant parts and was rather low in α -pinene. Tree 6 appeared to have higher β -phellandrene contents in most parts. Tree 7 is characterised by the highest myrcene contents (18.7%) in the cones. Trees 2 and 4 appeared to be most similar. High diversity of terpenes within a population might be seen in the context to increase resistance to herbivory (Keefover-Ring and Linhart 2010). Monoterpene variability occurred also within *Pinus sylvestris* where α -pinene and δ -3-carene rich chemotypes could be distinguished within the same populations (Kännaste et al. 2013). Accordingly, within a population, there were trees emitting mainly α -pinene

Table 2 Essential oil composition (%) and yield of needles and twig tips from the top twigs (NT) of *Pinus cembra*

Compound	RI	Needles (NT-N)		Twig tips (NT-T)	
		Mean	<i>SD</i>	Mean	<i>SD</i>
Oil yield (% v/w)		1.0	<i>0.4</i>	2.3	<i>0.4</i>
<i>Monoterpenes</i>					
α -Pinene	942	62.9	<i>6.0</i>	22.3	<i>5.1</i>
Camphene	957	1.2	<i>0.3</i>	2.0	<i>1.4</i>
Verbenene	962	0.5	<i>0.3</i>	0.1	<i>0.1</i>
β -Pinene	985	4.1	<i>2.3</i>	14.9	<i>6.0</i>
Myrcene	993	0.5	<i>0.2</i>	1.8	<i>0.1</i>
δ -3-Carene	1016	< 0.05	<i>0.0</i>	0.3	<i>0.1</i>
Limonene	1039	1.2	<i>1.5</i>	20.0	<i>12.2</i>
β -Phellandrene	1039	6.6	<i>5.6</i>	25.7	<i>7.2</i>
α -Terpineol	1197	0.5	<i>0.1</i>	0.5	<i>0.3</i>
Bornyl acetate	1293	0.3	<i>0.2</i>	1.3	<i>0.8</i>
Sum		77.8		88.9	
<i>Sesquiterpenes</i>					
α -Amorphene	1490	0.6	<i>0.3</i>	0.1	<i>0.1</i>
Germacrene D	1498	4.1	<i>1.7</i>	0.5	<i>0.3</i>
Bicyclogermacrene	1515	0.8	<i>0.3</i>	< 0.05	
γ -Cadinene	1527	0.9	<i>0.4</i>	0.2	<i>0.1</i>
δ -Cadinene	1537	1.8	<i>0.6</i>	0.4	<i>0.1</i>
Spathulenol	1596	1.0	<i>0.2</i>	< 0.05	
α -Cadinol	1671	1.1	<i>0.2</i>	0.1	<i>0.1</i>
α -Bisabolol	1697			0.2	<i>0.2</i>
Sum		10.3		1.5	
<i>Diterpenes</i>					
Cembrene	1955			0.5	<i>0.1</i>
Cembratrienol	2082	0.2	<i>0.1</i>	0.6	<i>0.4</i>
Abieta-6,8(14)-dien-18-al	2267			0.8	<i>0.3</i>
Methyl daniellate	2350	0.4	<i>0.2</i>	1.0	<i>0.7</i>
Sum		0.6		2.9	
Total sum		88.8		93.4	

Mean and standard deviation (*SD*) from 7 trees

Means are presented in ordinary fonts while standard deviations (*SD*) are given in italics

or δ -3-carene or a mixture of both compounds (Bäck et al. 2012).

Twig tips with needles were most commonly used to obtain the *P. cembra* essential oil in practice. In the present study, they came from the lower part of the tree (NB) and from the treetop (NT and NC). Their essential oil profiles were similar (Table 1; Fig. 2) with 43.9–48.3% α -pinene, 13.1–17.2% β -phellandrene and 6.6–9.3% β -pinene as main compounds. From the view of plant morphology these samples

consisted of two different plant organs, leaves and shoot tips. Secretory ducts are present in both parts but the amount and composition of the stored essential oil may be different. Therefore, the seven NT samples were further separated into needles (NT-N) and twig tips (NT-T) and analysed separately. The compositions of the resulting essential oils are presented in Table 2. Twig tips contained more essential oil (2.3%) than needles alone (1.0%). Needle essential oil contained considerably more α -pinene (62.9%) than

Fig. 2 **a** Projection of the plant parts from the different trees onto the plane of main PCA factors. **A:** factor 1 and factor 2, **b** factor 3 and factor 4. Description of abbreviations: Shoots and needles from the treetop (NT) the bottom (NB) and the cone bearing twigs (NC), one year old cones from treetop (CO), twigs without needles from the treetop (TT) and the bottom (TB), bark (BA), heartwood (HW) and sapwood (SW)

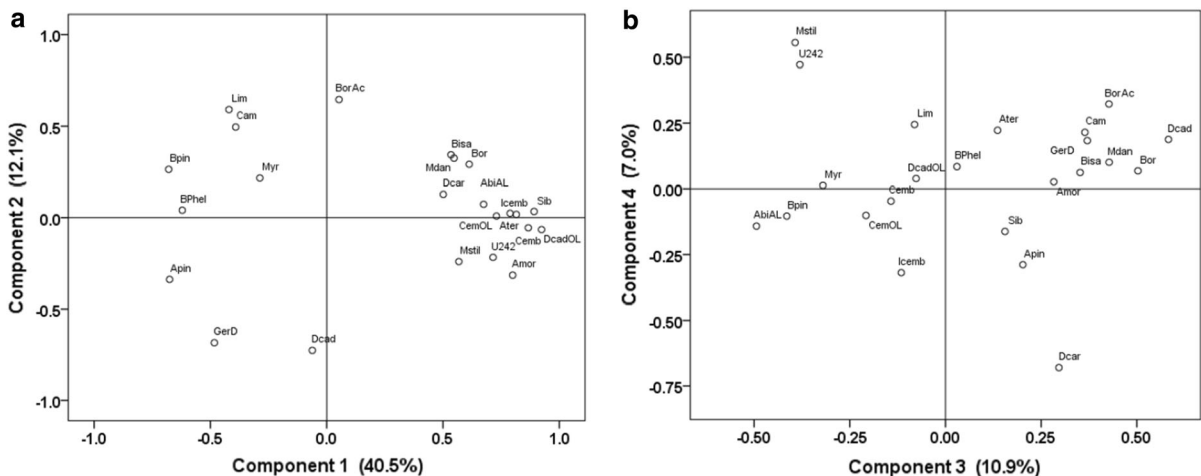
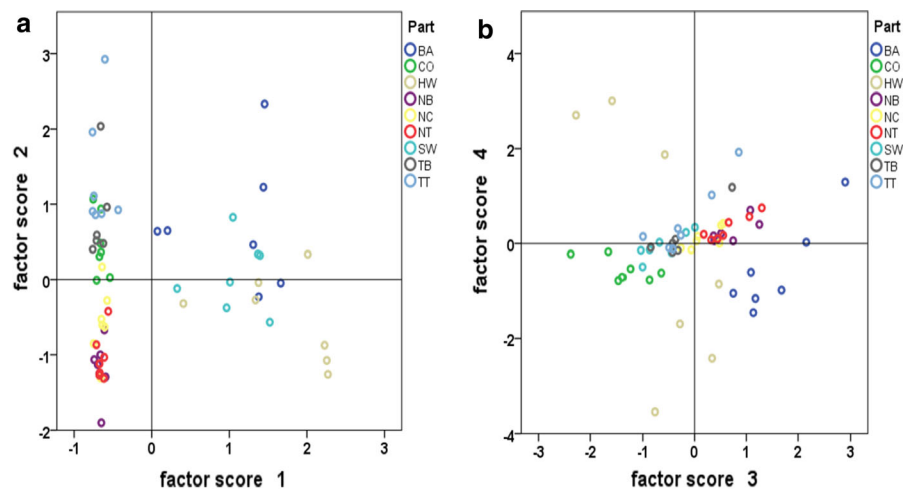


Fig. 3 Loading of the essential oil compounds on the components in PCA. **a** components 1 and 2, **b** components 3 and 4. The analysis included as variables: AbiAL: abieta-6,8(14)-dien-18-al, Amor: α -amorphene, Apin: α -pinene, Ater: α -terpineol, Bisa: α -bisabolol, Bor: borneol, BorAc: bornyl acetate, Bpin: β -pinene, Cam: camphene, Cemb: cembrene,

CemOL: cembratrienol, Dcad: δ -cadinene, DcaOL: δ -cadinol, Dcar: δ -3-carene, GerD: germacrene D, ICem: isocembrene, Lim: limonene, Mdan: methyl daniellate, MStil: 3,5-methoxystilbene, Myr: myrcene, BPhel: β -phellandrene, Sib: sibirene, U242: unidentified compound

twig tip essential oil (22.3%). A high percentage of α -pinene in *P. cembra* needle essential oil has also been reported by Apetrei et al. (2013) (69.1%) and Kubeczka and Schultze (1987) (64.7%). Currently, also the sesquiterpenes, in particular germacrene D, were at a higher level in the needles as compared to the twig tips. To counterbalance this, twig tip essential oil had higher β -phellandrene, limonene and β -pinene contents than the essential oils from the needles. Finally, more diterpenes were found in twig tips than in needles. In sum, the essential oil obtained from the

twig tips had a very similar composition to that obtained from the twigs up to 2 cm in diameter without needles (TT and TB). These results are in good accordance with data obtained from parts of *P. cembra* grown in an arboretum (Lis et al. 2017).

Volatiles from HS-SPME

Head space solid phase microextraction (HS-SPME) has been developed as fast solvent free extraction

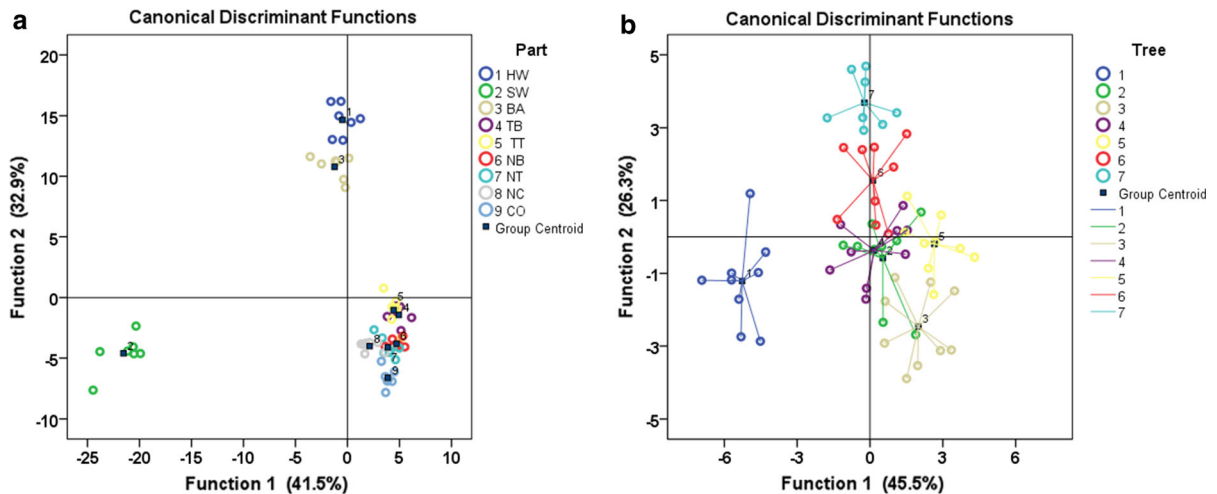


Fig. 4 **a** Discriminant scores of the two first functions grouping of the samples to the plant parts. Description of abbreviations: Plant parts as for Fig. 3. **b** Discriminant scores of the two first

functions grouping the samples to the individual trees; colours represent different trees, while dots represent the different plant parts

technique to isolate volatile compounds from a range of different plant matrices where the volatiles can be directly transferred into a GC-system. The headspace-SPME method has been applied to compare the volatiles from six *Pinus* species growing in Canada and the major compounds were suitable to be employed as chemotaxonomical markers (Kilic and Kocak 2014). Similarly, relative abundance of α -pinene, δ -3-carene and bornyl acetate in the headspace-SPME fraction from needles has been used to differentiate between the closely related species *Pinus mugo*, *P. uncinata* and *P. uliginosa* (Celinski et al. 2015).

In the present research, as shown in Table 3, α -pinene was the major volatile compound in all SPME fractions. High levels of this compound were also found in heartwood and sapwood, contrasting the distilled fractions where α -pinene was very low in some samples. Also *Pinus sylvestris* showed high α -pinene levels in SPME analyses of sapwood and heartwood (Wajs et al. 2007). Actually, in contrast to hydrodistillates, SPME fractions contained very few diterpenes. Therefore, SPME and distillates from wood (HW, SW) were clearly different. Alkane derivatives occurred also in sapwood SPME fractions with hexanoic acid and nonanal as major compounds. Furthermore, in contrast to distillates, SPME from twigs (TT) showed also an appreciable level (7.5%) of hexanoic acid (Table 3).

Currently, bark (BA) and wood (HW, SW) SPME fractions contained appreciable δ -3-carene amounts that were higher than in the respective essential oils. The high germacrene D levels in needles as compared to other tree parts could also be obtained in SPME. A comparison between essential oil composition and SPME is shown in the supplementary material (Figure S5) for the different parts of three trees. At least for needles (NT), cones (CO) and twigs (TT) the individual essential oil pattern of a tree could be observed as well in the corresponding SPME. So, in sum, SPME as an user-friendly, solvent-free and fast technique gave comparable results for the main volatile compounds in the case of cone and needle samples as compared to hydrodistillation. To a limited extent, this was also true for twigs but not for wood and bark samples.

Conclusions

The essential oils of *Pinus cembra* showed remarkable differences in their compositions between the different plant parts. Twig tips with needles and cones are rich in α -pinene, while twigs had also appreciable share of β -phellandrene, β -pinene and limonene in their essential oils. Twig tips with needles that are mostly used to obtain essential oil gave comparable essential oil compositions when harvested in different parts of the

Table 3 Composition of the volatile fractions (%) from the various plant parts of *Pinus cembra* analysed by HS-SPME/GC/FID

Compound	RI	NT-N		CO		TT		BA		HW		SW	
		Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>
<i>Monoterpenes</i>													
α -Pinene	925	62.9	<i>1.2</i>	43.1	<i>15.0</i>	26.2	<i>4.0</i>	34.2	<i>4.0</i>	55.9	<i>14.8</i>	20.3	<i>8.2</i>
Camphene	940	0.9	<i>0.2</i>	0.5	<i>0.1</i>	2.7	<i>2.4</i>	3.4	<i>2.3</i>	1.1	<i>0.5</i>	0.5	<i>0.3</i>
β -Pinene	969	2.1	<i>1.9</i>	26.2	<i>4.4</i>	12.1	<i>6.8</i>	5.7	<i>1.2</i>	2.1	<i>1.9</i>	4.5	<i>1.8</i>
Myrcene	980	0.5	<i>0.3</i>	8.9	<i>12.9</i>			0.5	<i>0.1</i>	0.5	<i>0.3</i>		
δ -3-Carene	1000	0.1	<i>0.0</i>	t		5.3	<i>6.7</i>	9.4	<i>0.6</i>	12.9	<i>1.6</i>	5.2	<i>3.8</i>
p-Cymene	1017	0.2	<i>0.1</i>	t		0.3	<i>0.0</i>	1.5	<i>1.1</i>	0.3	<i>0.2</i>	t	
Limonene	1023	1.5	<i>0.7</i>	10.4	<i>4.2</i>	5.2	<i>6.5</i>	4.4	<i>5.0</i>	2.7	<i>3.0</i>	0.2	<i>0.0</i>
β -Phellandrene	1023	3.3	<i>5.1</i>	7.6	<i>4.3</i>	17.4	<i>5.4</i>	7.6	<i>3.3</i>	0.6	<i>0.6</i>	1.2	<i>1.2</i>
Terpinolene	1082	0.3	<i>0.1</i>	0.2	<i>0.1</i>	0.6	<i>0.1</i>	0.9	<i>0.3</i>	1.2	<i>0.7</i>	0.3	<i>0.3</i>
α -Campholenal	1121	0.4	<i>0.2</i>	t		0.3	<i>0.1</i>	t		t		0.5	<i>0.2</i>
Borneol	1164	0.1	<i>0.1</i>	t		0.3	<i>0.1</i>	0.8	<i>0.4</i>	0.7	<i>1.0</i>	0.6	<i>1.1</i>
α -Terpineol	1188	0.1	<i>0.0</i>	0.3	<i>0.5</i>	t		0.9	<i>0.3</i>	3.9	<i>4.8</i>		
Thymol methyl ether	1232	0.1	<i>0.1</i>	0.1	<i>0.1</i>	1.3	<i>0.6</i>	1.3	<i>0.5</i>	0.2	<i>0.1</i>	0.2	<i>0.3</i>
Bornyl acetate	1287			0.2	<i>0.1</i>	4.0	<i>3.3</i>	0.8	<i>0.2</i>	0.3	<i>0.3</i>	1.4	<i>1.6</i>
Sum		72.5		97.5		75.7		71.4		82.4		34.9	
<i>Sesquiterpenes</i>													
Sibirene	1411	t		t		1.0	<i>0.9</i>	4.2	<i>2.2</i>	3.2	<i>2.4</i>	2.7	<i>1.3</i>
β -Caryophyllene	1430	1.0	<i>0.7</i>	0.1	<i>0.1</i>	1.6	<i>1.4</i>	1.0	<i>0.4</i>	0.8	<i>0.5</i>	0.7	<i>0.6</i>
α -Amorphene	1487	1.5	<i>0.5</i>	t		0.6	<i>0.4</i>	2.9	<i>1.3</i>	1.9	<i>1.4</i>	1.6	<i>1.2</i>
Germacrene D	1493	8.0	<i>2.5</i>	0.8	<i>0.9</i>	0.3	<i>0.4</i>	0.1	<i>0.2</i>	t			
α -Muurolene	1510			t		0.5	<i>0.5</i>	3.2	<i>1.5</i>	2.3	<i>1.5</i>	1.9	<i>0.7</i>
γ -Cadinene	1527	2.2	<i>0.8</i>	t		0.3	<i>0.2</i>	1.1	<i>0.4</i>	0.2	<i>0.1</i>		
δ -Cadinene	1537	2.6	<i>0.7</i>	t		0.2	<i>0.3</i>	1.8	<i>0.4</i>	1.0	<i>0.6</i>	0.5	<i>0.3</i>
Sum		15.5		0.9		4.5		14.3		9.4		7.4	
<i>Diterpenes</i>													
Cembrene	1960							0.2	<i>0.1</i>	0.7	<i>0.6</i>		
Alkane derivatives													
Hexanoic acid	981					7.5	<i>5.5</i>					21.6	<i>6.8</i>
n-Octanal	1003					0.3	<i>0.2</i>					2.6	<i>0.7</i>
n-Nonanal	1105					0.4	<i>0.3</i>			0.1	<i>0.1</i>	4.5	<i>0.4</i>
Octanoic acid	1175											1.7	<i>1.7</i>
Nonanoic acid	1275					0.3	<i>0.2</i>					1.9	<i>1.0</i>
Methyl decanoate	1322	0.2	<i>0.1</i>					1.2	<i>1.6</i>				
Sum		0.2				8.5		1.2		0.1		32.3	
Total Sum		88.0		98.4		88.7		87.0		92.5		74.7	

t: traces (< 0.05%)

Needles from the top twigs (NT-N), one year old cones from treetop (CO), twigs without needles from the treetop (TT), bark (BA), heartwood (HW) and sapwood (SW)

Means are presented in ordinary fonts while standard deviations (SD) are given in italics

crown. Material from the trunk is low in essential oil and contained mainly diterpenes, some monoterpenes and alkane derivatives. A future evaluation of the biological activities of the essential oils must consider these differences in composition of the volatile fractions.

Supplementary materials

The following are available online: Table S1: Essential oil yield and composition (%) obtained from the different parts of *Pinus cembra*; Figure S1: Mean essential oil composition in the different plant parts of *Pinus cembra*; Figure S2: Essential oil composition of needles with twigs in the crown from the top (NT), the bottom (NB) and cone bearing twigs (NC) of seven individual trees; Figure S3: Essential oil composition of cones (CO), twigs from the top (TT) and the bottom (TB) of the crown of seven individual trees; Figure S4: Essential oil composition of bark (BA), heartwood (HW) and sapwood (SW) of seven individual trees; Figure S5: Comparison of volatile compounds composition in distillates and SPME-fractions obtained from different parts of *Pinus cembra*.

Acknowledgements We are thankful to Bettina Bein-Lobmaier and Harry Bein for carrying out the analyses. Special thanks to Erwin Korbuly and his sons Peter and Clemens for their great support by organizing the transport to the location of the *Pinus cembra* trees, for cutting the trees and for providing the plant materials.

Author Contributions Conceptualization, KM; methodology, KM and RC; data curation, RC; writing—original draft preparation, RC; writing—review and editing, KM. Both authors agreed with the final version of the manuscript.

Funding Open access funding provided by University of Veterinary Medicine Vienna.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors declare that no ethical approval was needed for this research. No humans or animals have been a subject of this research.

Human and animal rights This article does not contain any studies with human or animal subjects.

Informed consent Both authors approved the final version of the manuscript.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Adams RP (2007) Identification of essential oil components by gas chromatography/quadrupole mass spectrometry, 4th edn. Allured, Carol Stream
- Apetrei CL, Spac A, Brebu M, Tuchilus C, Miron A (2013) Composition, and antioxidant and antimicrobial activities of the essential oils of a full-grown *Pinus cembra* L. tree from the Calimani Mountains (Romania). J Serbian Chem Soc 78(1):27–37. <https://doi.org/10.2298/JSC120409075A>
- Apetrei CL, Tuchilus C, Aprotosoae AC, Oprea A, Malterud KE, Miron A (2011) Chemical, antioxidant and antimicrobial investigations of *Pinus cembra* L. barks and needles. Molecules 16:7773–7788. <https://doi.org/10.3390/molecules16097773>
- Bäck J, Aalto J, Henriksson M, Hakola H, He Q, Boy M (2012) Chemodiversity of a scots pine stand and implication for air terpene concentrations. Biogeosciences 9:689–702. <https://doi.org/10.5194/bg-9-689-2012>
- Boden S, Pyttel P, Eastaugh CS (2009) Impacts of climate change on the establishment, distribution, growth and mortality of Swiss stone pine (*Pinus cembra* L.). iForest 3:82–85
- Caudullo G, de Rigo D (2016) *Pinus cembra* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz J, de Rigo D, Caudullo G, Houston Durrant T, Mauri A. European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, e01bd9b
- Celinski K, Bonikowski R, Wojnicka-Poltorak A, Chudzinska E, Malinski T (2015) Volatiles as chemosystematic markers for distinguishing closely related species within the *Pinus mugo* complex. Chem Biodivers 12:1208–1213
- Chizzola R, Lohwasser U, Franz Ch (2018) Biodiversity within *Melissa officinalis*: Variability of Bioactive Compounds in a Cultivated Collection. Molecules 23:294. <https://doi.org/10.3390/molecules23020294>
- Domrachev DV, Karpova EV, Goroshkevich SN, Tkachev AV (2012) Comparative analysis of volatiles from needles of

- five-needle pines of northern and eastern Eurasia. *Rus J Bioorg Chem* 38(7):780–789
- Dormont L, Roques A, Malosse C (1998) Cone and foliage volatiles emitted by *Pinus cembra* and some related conifer species. *Phytochemistry* 49(5):1269–1277
- Ducci F, Cantiani P, Cutini A, Dettori S (2014) Forests and non wooden products in Italy. Proceedings of the Second International Congress of Silviculture (Florence, November, 26th–29th 2014): 650–657
- Graikou K, Gortzi O, Mantanis G, Chinou I (2012) Chemical composition and biological activity of the essential oil from the wood of *Pinus heldreichii* Christ. var. *leucodermis* Eur J Wood Products 70:615–620. <https://doi.org/10.1007/s00107-012-0596-9>
- Höhn M, Gugerli F, Abran P, Bisztray G, Buonamici A, Cseke K, Hufnagel L, Quintela-Sabaris C, Sebastiani F, Vendramin GG (2009) Variation in the chloroplast DNA of Swiss stone pine (*Pinus cembra* L.) reflects contrasting post-glacial history of populations from the Carpathians and the Alps. *J Biogeogr* 36:1798–1806
- Ioannou E, Koutsaviti A, Tzakou O, Roussis V (2014) The genus *Pinus*. A comparative study on the needle essential oil composition of 46 pine species. *Phytochem Rev* 13(4):741–768. <https://doi.org/10.1007/s11101-014-9338-4>
- Kännaste A, Copolovici L, Pazouki L, Suhhorutsenko M, Niinemets Ü (2013) Highly variable chemical signatures over short spatial distances among Scots pine (*Pinus sylvestris*) populations. *Tree Physiol* 33:374–387. <https://doi.org/10.1093/treephys/tp013>
- Keefover-Ring K, Linhart YB (2010) Variable chemistry and herbivory of ponderosa pine cones. *Int J Plant Sci* 171(3):293–302. <https://doi.org/10.1086/650155>
- Kilic Ö, Kocak A (2014) Essential Oil Composition of Six *Pinus* L. Taxa (Pinaceae) from Canada and Their Chemotaxonomy. *J Agric Sci Technol B* 4:67–73
- Kubeczka KH, Schultz W (1987) Biology and Chemistry of Conifer Oils. *Flavour Fragrance J* 2:137–148
- Land T (2018) Bericht über die Lager der Tiroler Land- und Forstwirtschaft. *Grüner Bericht 2018*. Land Tirol, Amt der Tiroler Landesregierung, Innsbruck
- Landwirtschaftskammer Tirol (2014) Tirols Land- und Forstwirtschaft in Zahlen. Landwirtschaftskammer Tirol, Innsbruck
- Lis A, Kalinowska A, Krajewska A, Mellor K (2017) Chemical Composition of the Essential Oils from Different Morphological Parts of *Pinus cembra* L. *Chem Biodivers* 14(4):e1600345. <https://doi.org/10.1002/cbdv.201600345>
- McLafferty FW (1989) Registry of mass spectral data, 5th edn. Wiley, New York
- Nikolic B, Todosijevic M, Ratknic M, Dordevic I, Stankovic J, Cvetković M, Marin PD, Tesevic V (2018) Terpenes and n-Alkanes in Needles of *Pinus cembra*. *Nat Prod Commun* 13(8):1035–1037
- Ochocka JR, Asztemborska M, Sybilka D, Langa W (2002) Determination of enantiomers of terpenic hydrocarbons in essential oils obtained from species of *Pinus* and *Abies*. *Pharmaceut Biol* 40(5):395–399. <https://doi.org/10.1076/phbi.40.5.395.8452>
- Schütt P (2014) *Pinus Cembra*. In: Roloff A, Weissgerber H, Lang UM, Stimm B *Enzyklopädie der Holzgewächse*. Wiley – VCH, Weinheim, 12 pp
- Schweingruber FHZ, Wirth C (2009) Old Trees and the Meaning of „Old“. In: Wirth C (2009) *Old-Growth Forests, Ecological Studies* 207, Springer, Berlin, pp. 35–54
- Smith JP (2017) Economically Important Plants Arranged Systematically. *Bot Studies* 48:1–33
- Thaler-Rizzoli S, Gasteiger H (2017) Die Zirbe. *Athesia, Bolzano*, pp 136–137
- Venditti A, Serrilli AM, Vittori S, Papa F, Maggi F, Di Cecco M, Ciaschetti G, Bruno M, Rosselli S, Bianco A (2013) Secondary metabolites from *Pinus mugo* Turra subsp. *mugo* growing in the Majella Park (Central Apennines, Italy). *Chem Biodivers* 10:2091–2100
- Wajs A, Pranovich A, Reunanen M, Willför S, Bjarne H (2007) Headspace SPME analysis of the sapwood and heartwood of *Picea abies*, *Pinus sylvestris* and *Larix decidua*. *J. Essent. Oil Res.* 19: 125–133. <https://doi.org/10.1080/10412905.2007.9699244>
- Weiss G, Ludvig A, Zivojinovic I, Asamer-Handler M, Huber P (2017) Non-timber innovations: How to innovate in side-activities of forestry – Case study Styria, Austria. *Austrian J Forest Sci* 134(Special Issue 1a):231–250
- Wieser G, Manning WJ, Tausz M, Bytnerowicz A (2006) Evidence for potential impacts of ozone on *Pinus cembra* L. at mountain sites in Europe: An overview. *Environ Pollut* 139:53–58
- Wojnicka-Póltorak A, Celiński K, Chudzińska E, Prus-Głowacki W, Niemtur S (2015) Genetic Resources of *Pinus cembra* L. Marginal Populations from the Tatra Mountains: Implications for Conservation. *Biochem Genet* 53:49–61
- Zong C, Wauters LA, Van Dongen S, Mari V, Romeo C, Martinoli A, Preatoni D, Guido T (2010) Annual variation in predation and dispersal of Arolla pine (*Pinus cembra* L.) seeds by Eurasian red squirrels and other seed-eaters. *Forest Ecol Managem* 260:587–594

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.