Intraspecific Variability of Self-sown Scots Pine (*Pinus sylvestris* L.) Occurring in Eastern Poland in Respect of Essential Oil Content and Composition

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Abstract

The aim of this study was to investigate the chemical variability between self-sown Scots pine (*Pinus sylvestris* L.) populations, grouped according to Polish Forest Growth Regions. Plant material (shoot buds and needles) was evaluated in respect of the content and composition of essential oil. The total content of essential oil was determined by hydrodistillation, while its qualitative and quantitative analysis was performed by GC/MS and GC/FID. The major compounds in both raw materials were ä-3-carene and į-pinene. High content of ä-3-carene (8.78 – 9.25%) in needle essential oils among all the populations indicate that they belong to ä-3-carene chemotype. The accumulation of essential oil (0.65-1.45% in shoot buds; 0.83-0.96% in needles), as well as its composition were associated with latitude. Populations localized in the northernmost part of Poland (Mazury-Podlasie Region) were distinguished by the highest content of essential oil as well as the highest share of į-pinene and ā-pinene, both in shoot buds and needles.

Key words: Scots pine, biodiversity, herbal raw materials, essential oils, forest growth regions

Introduction

Scots pine (*Pinus sylvestris* L.) is a long-lived coniferous tree of high economic significance, utilized mainly for its timber and bark (Witkowska-Żuk 2008). Moreover, this is a valuable medicinal and aromatic plant providing different herbal materials, i.e.: shoot buds (*Pini Gemmae*), young shoots (*Pini Turiones*), needles (*Pini Folium*) and essential oil (*Pini Oleum*) obtained by hydrodistillation from these raw materials (Strzelecka and Kowalski 2000). In Poland, shoot buds and needles are collected in winter and early spring mainly from self-sown Scots pine trees as well as from branches of mature trees, which are a waste product at forest clearing and maintenance works such as glades or thinning. The most valuable raw materials are obtained from trees not older than ten years, growing on ecologically clean areas (Góra and Lis 2012, Staniszewski 2013). Shoot buds and needles are rich in essential oil containing predominantly monoterpenes: α-pinene, β-pinene, δ-3-carene, β-myrcene, limonene, β-phellandrene, p-cymene, bornyl acetate, α-terpinolene and sesquiterpenes: β-caryophyllene, germacrene D, γ-cadinene and δ-cadinene (Orav et al. 1996, Venskutonis et al. 2000, Sjödin et al. 2000, Judzentiene et al. 2006, Thoss et al. 2007, Kupcinskiene et al. 2008, Judzentiene and Kupcinskiene 2008, Tümen et al. 2010, Kędzia et al. 2012). Diterpene resinous acids, juniperic and *sabinic* acid derivatives, tannins, bitter compounds and vitamin C have also have been detected (Strzelecka and Kowalski 2000). The needle essential oil is listed in European Pharmacopeia 8th edition. Extracts from the shoot buds, young shoots and needles reveal various pharmacological activities, i.e.: expectorant, antispasmodic, antimicrobial, warming, diaphoretic, diuretic and cholagogue. They are used in the prevention and treatment of upper respiratory tract rhinitis, in cough and inflammation of the throat and oral cavity, in the treatment of stomach, intestines and urinary tract diseases and externally as the antimicrobial and warming agents. Among herbal raw materials collected from Scots pine, shoot buds are the most popular in Poland, due to their utilization in production of syrup *Pini Compositus*, which indicates expectorant, antispasmodic and antimicrobial activity. Pine essential oil is also utilized in cosmetics and perfumery industry (Strzelecka and Kowalski 2000, Krauze-Baranowska et al. 2002, Motiejūnaitė and Pečiulytė 2004, Kędzia and Kędzia 2009, Kohlmūnzer 2010, Góra and Lis 2012, Kędzia et al. 2012, European Pharmacopeia 8th).

In Poland, Scots pine covers about 68% of the forests area. It is the main species in all habitats of coniferous forests as well as a species that is co-dominant with oak and beech in the mixed fresh forests (Witkowska-Żuk 2008, Matuszkiewicz 2011). Moreover, it is also planted in managed forests, in the form of solid monoculture stands. Due to the different climatic and physiographic conditions resulting from the range of glaciations, 8 Forest Growth Regions were distinguished in Poland. In these areas, the main tree species, especially Scots pine, are differentiated in terms of construction of treestands and their suitability for forestry production (Zielony and Kliczkowska 2012). In Poland, forests are usually surrounded by wastelands excluded from any silvicultural or agricultural treatment. Such areas, located close to the forest boundaries, are places where Scots pine appears in the early stages of secondary succession as a pioneering plant (Buraczyk 2013). Numerous wild growing self-sown trees, which represent a natural regeneration of the species, are the abundant source of herbal raw materials, especially shoot buds.

The content and composition of essential oil from plants that belong to *Pinus* genus can be related both to the genetic (Hiltunen 1976, Pohjola et al. 1989) and environmental factors, such as temperature, intensity of solar radiation (Lincoln and Langheim 1978, Gleizes et al. 1980), day length (Yazdani and Nilsson 1986) and humidity (Baţer and Bouchbauer 2009). According to Hanover (1992) and Forrest (1980), the composition of essential oils in conifers may be used as an indicator in the study of biodiversity: genetic affinity, intraspecific variability or a fingerprint-type analysis. The composition of needle essential oil was investigated by many authors (Orav et al. 1996, Venskutonis et al. 2000, Judzentiene et al. 2006, etc.). However, data concerning shoot buds, a younger developmental stage of needles, are rather scare. The chemical composition of pine essential oil may be significantly influenced by geographical location (Muona et al. 1986, Venskutonis et al. 2000). One of the first population studies of terpenes from Scots pine needles were carried out by Naydenov et al. (2002, 2005). The studies conducted in Finland, Estonia, Lithuania, Bulgaria and Turkey show that the selected ecotypes of Scots pine differ in content and composition of terpenes (Manninen et al. 1998, Venskutonis et al. 2000, Manninen et al. 2002, Naydenov et al. 2002 and 2005, Semiz et al. 2007).

Although Scots pine is the dominant element of forest stands in Poland and provides valuable medicinal and aromatic raw materials, there is little information on the chemical diversity within this species in this region. The aim of this study was to determine the chemical variability of Scots pine self-sown trees occurring on the wastelands surrounding forests in the eastern area of Poland, in terms of content and composition of essential oil in shoot buds and needles from one-year-old shoots, taking into account the forest regionalization.

Materials and Methods

Geographical localization of Scots pine self-sown populations

The study covered the area of eastern Poland where 22 natural sites of self-sown Scots pine populations were selected. Their geographical coordinates are provided in Table 1. According to the forest regionalization (Zielony and Kliczkowska 2012), the area of the research covers three Polish Forest Growth Regions: Mazury-Podlasie (II), Mazovia-Podlasie (IV) and Malopolska (VI) (Table 1, Figure 1).

Plant samples

The research was carried out in 2014. Plant material – shoot buds and needles from one-year-old shoots of Scots pine – were collected from wild growing young (7-12-year-old) self-sown trees (Figure 2). They occurred close to forests, mainly on abandoned agricultural lands, located not more than 400 m from mature trees stands (the distance from a source of seeds). Collection of shoot buds and one-year-old needles was carried out in February, before beginning of plant vegetation (Figure 3). At each site, the plant material was collected from minimum ten pine trees, and then mixed to obtain a sample representative for population. Voucher specimens were deposited at the herbarium of the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences, WULS-SGGW, Poland. Shoot buds and needles were dried at 35°C and subjected to chemical evaluation concerning the content and composition of essential oil.

Table 1. Geographical localization of Scots pine self-sown populations

Population No.	Latitude	Longitude	Altitude	Polish Forest Growth Regions*
1	N 52.8246	E 23.7332	169	II–Mazury-Podlasie
2	N 52.8490	E 23.4354	152	II–Mazury-Podlasie
3	N 52.7451	E 23.5816	162	II–Mazury-Podlasie
4	N 53.2037	E 21.5951	115	II–Mazury-Podlasie
5	N 53.0837	E 22.1112	176	II–Mazury-Podlasie
6	N 53.1825	E 22.0145	108	II–Mazury-Podlasie
7	N 52.1958	E 21.3477	98	IV- Mazovia-Podlasie
8	N 52.2123	E 21.4083	100	IV- Mazovia-Podlasie
9	N 52.1102	E 21.5213	99	IV- Mazovia-Podlasie
10	N 52.0251	E 20.2153	113	IV- Mazovia-Podlasie
11	N 52.5112	E 20.0033	121	IV- Mazovia-Podlasie
12	N 52.1247	E 21.4991	137	IV- Mazovia-Podlasie
13	N 52.2978	E 21.5545	87	IV- Mazovia-Podlasie
14	N 52.1455	E 21.5289	117	IV- Mazovia-Podlasie
15	N 50.3870	E 22.4450	223	VI – Malopolska
16	N 50.3648	E 22.5253	203	VI – Malopolska
17	N 50.3508	E 22.5608	197	VI – Malopolska
18	N 50.3798	E 22.5063	190	VI – Malopolska
19	N 50.3971	E 22.5226	195	VI – Malopolska
20	N 50.4795	E 22.4095	166	VI – Malopolska
21	N 50.5602	E 22.3705	199	VI – Malopolska
22	N 50.5451	E 22.2745	197	VI – Malopolska

*Polish Forest Growth Regions are shown on Figure 1

Figure 1. The map of Poland with Mazury-Podlasie (II), Mazovia-Podlasie (IV) and Malopolska (VI) Forest Growth Regions marked

Figure 2. Natural stand of self-sown Scots pine on the boundaries of forest (population no. 5).

Figure 3. Scots pine shoot buds

Chemical analysis

The content of essential oil was evaluated using hydrodistillation in Clevenger apparatus for 3 h, according to European Pharmacopeia 8th edition. The volume of 500 ml distilled water was added to 30 g of raw materials. Obtained essential oils were stored in dark vials, at 4°C.

The qualitative GC/MS analysis were carried out using Shimadzu GC/MS QP210S gas chromatograph equipped with Phenomenex Zebron ZBFFAP polar column (30 m × 0.25 mm × 0.25 µm film thickness). The operating conditions were as follows: oven temperature was isothermal at 60°C within 2 min., then rising at 4°C per min. to 210°C and held isothermal for 5 min. Injector temperature: 210°C. The carrier gas (He) flow was 1.1 ml × min^{-1}. The split ratio was 1:50. Diluted samples (1/100 v/v, in *n*-hexane:isopropanol) of 1 µl were injected at 210°C by autosampler. Ion source temperature -220°C, ionization voltage 70 eV. Mass spectra were scanned in the range of 40-500 amu. Essential oil compounds identification was based on comparison of mass spectra from the Mass Spectral Database as follows: NIST08, NIST27, NIST147, Wiley7N2, PAL 600, and on comparison of retention indices (RI) relative to retention times of a series of n-hydrocarbons (C_7–C_{30}) with those reported in literature.

The quantitative GC/FID analysis were performed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and capillary, polar column HP 20M (25 m × 0.32 mm × 0.30 µm film thickness). The analysis was carried out using the following temperature programme: oven temperature was isothermal at 60 °C for 2 min., then it was programmed from 60 °C to 220 °C at a rate of 4 °C per min. and held isothermal at 220 °C for 5 min. Injector and detector temperatures were at 220 °C and 260 °C, respectively. The carrier gas (He) flow was 1.1 ml × min^{-1}. The split ratio was 1:150. Manually, injection of 0.1 µl pure essential oil. Component identification was confirmed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (RI) relative to the series of *n*-hydrocarbons (C_7-C_{30}) under the same operating conditions. Retention indices of compounds were also compared with those reported in the literature.

The percentage composition of the essential oils was computed by the normalization method from the GC peak areas without the use of correction factors. All the analyses were performed in triplicate.

Statistical analysis

All results were subjected to statistical analysis using Statistica® software and expressed as means values ± standard deviation (*SD*) of population groups located in Polish Forest Growth Regions. The mean values were compared by using the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The differences between individual means were deemed to be significant at $P < 0.05$ and were marked as different letters in column rows.

Results

Investigated populations of self-sown Scots pine occurring in eastern Poland were grouped according to forest regionalization. As a result, three units were obtained as follows: populations located in Malopolska, Mazovia-Podlasie and Mazury-Podlasie Regions (Table 1, Figure 1). Results of the present work indicate that these groups of populations differ in respect of essential oil content and composition in shoot buds and needles.

Essential oil content, in both examined raw materials increased with increasing latitude. The lowest content of essential oil was noted in the most southerly located populations, in Malopolska (0.65% in shoot buds and 0.83% in needles), slightly higher – in populations from Mazovia-Podlasie (1.06% in shoot buds, 0.94% in needles), and the highest in the northernmost populations on Mazury-Podlasie (1.45%; 0.96%, respectively). Observed differences were significant only in the case of shoot bud essential oils. Shoot buds were characterized by higher content of essential oil (1.03%) in comparison to needles (0.93%) (Table 2).

In the case of shoot buds, 30 compounds were detected in essential oil, what comprised 93.42-94.65% of the samples. The monoterpene hydrocarbons fraction was the fundamental part in the essential oil, since it formed 73.95% (Malopolska) – 78.06% (Mazury-Podlasie) of the essential oils. Here, the share of monoterpene hydrocarbons increased with increasing latitude, in opposite to oxygenated monoterpenes. The dominant compound was δ-3-carene (38.23% in Mazury-Podlasie; 40.11% in Mazovia-Podlasie and 40.15% in Malopolska) followed by α-pinene (14.17; 12.02; 12.59%, respectively). The limonene and β-pinene was also present in considerable amounts. It is worth noting that the content of limonene increased with increasing latitude. Oxygenated monoterpenes fraction comprised 14.96% - 17.82% of the essential oils. It was represented primarily by p-cymen-8-ol, geraniol, α-phellandrenol and terpinen-4-ol. The share of sesquiterpenes, both hydrocarbons and oxygenated compounds was rather low (Table 3).

Table 2. Total content of essential oil in shoot buds and needles (%)

	Polish Forest Growth Regions			
	II Mazury-Podlasie (n=6)	IV Mazovia-Podlasie (n=8)	VI Malopolska (n=8)	Grand mean
Shoot buds	1.45 ± 0.53 a	1.06 ± 0.26 b	0.65 ± 0.04 c	1.03 ± 0.21
Needles	0.96 ± 0.25	0.94 ± 0.06	0.83 ± 0.07	0.93 ± 0.18

$P < 0.05$; n = number of populations

Table 3. The composition of essential oil in shoot buds (%)

			Polish Forest Growth Regions		
No.	Compound	RI*	II Mazury-Podlasie (n=6)	IV Mazovia-Podlasie (n=8)	VI Malopolska (n=8)
1	α-thujene	1013	0.22 ± 0.11 b	0.26 ± 0.09 ab	0.33 ± 0.04 a
2	α-pinene	1028	14.17 ± 1.84	12.02 ± 2.81	12.59 ± 1.61
3	camphene	1088	0.40 ± 0.06	0.37 ± 0.12	0.35 ± 0.03
4	β-pinene	1113	8.26 ± 3.96	7.16 ± 2.23	7.86 ± 2.23
5	sabinene	1124	0.76 ± 0.24	0.73 ± 0.12	0.76 ± 0.10
6	δ-3-carene	1151	38.23 ± 1.43	40.11 ± 5.97	40.15 ± 2.94
7	β-myrcene	1166	2.49 ± 0.93	2.04 ± 0.98	1.66 ± 0.47
8	*trans*-3-caren-2-ol	1175	1.03 ± 0.62 b	1.50 ± 0.22 a	1.69 ± 0.34 a
9	limonene	1203	8.61 ± 3.25 a	7.44 ± 2.37 ab	5.03 ± 1.21 b
10	β-phellandrene	1207	2.26 ± 0.76	1.71 ± 0.51	2.01 ± 0.60
11	ocimene	1265	1.79 ± 0.49	2.13 ± 0.35	1.92 ± 0.31
12	α-terpinolene	1278	0.88 ± 0.43 b	0.66 ± 0.38 b	1.31 ± 0.28 a
13	limonene oxide	1426	0.31 ± 0.10	0.37 ± 0.07	0.28 ± 0.06
14	yunipene	1557	0.44 ± 0.13	0.32 ± 0.30	0.42 ± 0.08
15	bornyl acetate	1576	0.65 ± 0.33	0.70 ± 0.32	0.49 ± 0.12
16	terpinen-4-ol	1596	1.59 ± 0.48	1.64 ± 0.30	1.62 ± 0.18
17	myrtenal	1608	0.36 ± 0.08	0.38 ± 0.11	0.36 ± 0.05
18	pinocarveol	1635	0.80 ± 0.20	0.82 ± 0.32	0.91 ± 0.11
19	isoborneol	1658	0.32 ± 0.04 b	0.42 ± 0.10 ab	0.46 ± 0.09 a
20	α-terpineol acetate	1677	1.17 ± 0.29	1.34 ± 0.37	1.30 ± 0.19
21	α-terpineol	1681	0.84 ± 0.25	1.03 ± 0.42	0.95 ± 0.14
22	a-phellandrenol	1696	1.62 ± 0.50 b	1.36 ± 0.64 b	2.90 ± 0.55 a
23	carvone	1711	0.80 ± 0.21 b	0.60 ± 0.31 b	1.39 ± 0.56 a
24	germacrene B	1725	0.39 ± 0.11	0.54 ± 0.19	0.46 ± 0.11
25	γ-cadinene	1793	0.52 ± 0.30	0.47 ± 0.15	0.43 ± 0.15
26	carveol	1810	0.43 ± 0.12	0.45 ± 0.20	0.37 ± 0.05
27	p-cymen-8-ol	1819	3.07 ± 1.20	4.13 ± 1.40	3.50 ± 0.94
28	geraniol	1826	1.78 ± 0.60	2.35 ± 0.69	1.84 ± 0.42
29	verbenone	1884	0.26 ± 0.20	0.38 ± 0.26	0.15 ± 0.07
30	caryophyllene oxide	1895	0.39 ± 0.34	0.40 ± 0.17	0.25 ± 0.08
	Monoterpene hydrocarbons		78.06 ± 2.23	74.56 ± 7.93	73.95 ± 3.79
	Oxygenated monoterpenes		14.96 ± 3.27	17.34 ± 3.60	17.82 ± 2.25
	Sesquiterpene hydrocarbons		1.24 ± 0.29	1.15 ± 0.55	1.31 ± 0.18
	Oxygenated sesquiterpenes		0.39 ± 0.34	0.39 ± 0.17	0.25 ± 0.08
	Total identified		94.65	93.42	94.55

$P < 0.05$; *RI on polar column (Zebron ZBFFAP, Phenomenex); n = number of populations.

Given the essential oil from needles, 39 compounds were identified, accounting for 87.04-90.32% of the samples. Similarly, to shoot bud essential oils, the main fraction was monoterpene hydrocarbons, which comprised 51.28% (Mazovia-Podlasie) – 57.17% (Mazury-Podlasie) of essential oils. Here, the clear dominant was α-pinene (36.86% in Mazury-Podlasie; 31.01% in Mazovia-Podlasie and 33.26% in Malopolska). Within this fraction, δ-3-carene, camphene and β-pinene were present in high amounts as well. In contrary to shoot bud essential oil, the content of sesquiterpenes, both hydrocarbons and oxygenated compounds was significantly higher here. Sesquiterpene hydrocarbons content decreased with increasing latitude and was at a level of 14.48% in Mazury-Podlasie, 17.44% in Mazovia-Podlasie and 21.06% in Malopolska. The share of oxygenated sesquiterpenes was slightly lower (from 11.43% in Malopolska to 13.95% in Mazovia-Podlasie). Sesquiterpenes were represented mainly by δ-cadinene (hydrocarbone) and α-cadinol (oxygenated compound) (Table 4).

Table 4. The composition of essential oil in needles (%)

No.	Compound	RI*	Polish Forest Growth Regions		
			II Mazury-Podlasie (n=6)	IV Mazovia-Podlasie (n=8)	VI Malopolska (n=8)
1	α-thujene	1013	1.19 ± 0.16	1.09 ± 0.13	1.11 ± 0.12
2	α-pinene	1028	36.86 ± 2.29	31.01 ± 5.72	33.26 ± 1.77
3	camphene	1088	4.91 ± 0.65	4.85 ± 0.58	4.98 ± 0.66
4	β-pinene	1113	2.55 ± 0.07	2.38 ± 0.54	2.27 ± 0.18
5	sabinene	1124	0.42 ± 0.07	0.40 ± 0.05	0.37 ± 0.04
6	δ-3-carene	1151	8.81 ± 2.20	9.25 ± 1.82	8.78 ± 1.30
7	β-myrcene	1166	1.30 ± 0.07 b	1.34 ± 0.10 b	1.66 ± 0.12 a
8	limonene	1203	0.62 ± 0.37	0.38 ± 0.13	0.50 ± 0.06
9	γ-terpinene	1248	0.20 ± 0.13 b	0.29 ± 0.11 b	0.59 ± 0.01 a
10	ocimene	1265	0.27 ± 0.05	0.28 ± 0.09	0.18 ± 0.01
11	α-terpinolene	1278	0.16 ± 0.01 b	0.30 ± 0.15 b	0.70 ± 0.25 a
12	α-cubebene	1445	0.14 ± 0.01	0.15 ± 0.03	0.10 ± 0.01
13	α-copaene	1480	0.25 ± 0.06	0.27 ± 0.05	0.19 ± 0.01
14	bourbonene	1536	0.09 ± 0.01	0.12 ± 0.02	0.26 ± 0.18
15	bornyl acetate	1576	2.44 ± 2.07	2.20 ± 0.44	2.17 ± 1.06
16	β-caryophyllene	1583	2.38 ± 0.39	2.50 ± 0.36	2.57 ± 2.53
17	aromadendrene	1598	0.22 ± 0.06	0.19 ± 0.04	0.18 ± 0.04
18	β-terpineol	1622	0.14 ± 0.01	0.15 ± 0.03	0.13 ± 0.02
19	α-humulene	1657	0.48 ± 0.21	0.62 ± 0.29	0.56 ± 0.01
20	α-terpineol acetate	1677	0.59 ± 0.06 ab	0.72 ± 0.08 a	0.54 ± 0.04 b
21	α-terpineol	1681	0.38 ± 0.14	0.41 ± 0.09	0.41 ± 0.19
22	germacrene D	1698	1.01 ± 0.61 b	0.93 ± 0.48 b	2.04 ± 0.33 a
23	β-selinene	1714	0.60 ± 0.15	0.75 ± 0.13	0.58 ± 0.04
24	α-selinene	1720	1.54 ± 0.19	1.85 ± 0.23	1.52 ± 0.07
25	γ-elemene	1728	0.92 ± 0.64 b	1.04 ± 0.31 b	2.89 ± 0.03 a
26	δ-cadinene	1769	7.00 ± 1.89 b	8.87 ± 1.32 ab	10.00 ± 1.48 a
27	α-muurolene	1786	0.17 ± 0.06	0.21 ± 0.04	0.12 ± 0.04
28	calamenene	1815	0.25 ± 0.03 b	0.31 ± 0.04 b	0.27 ± 0.04 ab
29	Nerol	1795	0.11 ± 0.03	0.12 ± 0.03	0.11 ± 0.01
30	Carveol	1810	0.11 ± 0.01	0.13 ± 0.02	0.16 ± 0.02
31	p-cymen-8-ol	1819	0.29 ± 0.09 ab	0.38 ± 0.08 a	0.19 ± 0.01 b
32	geraniol	1826	0.20 ± 0.01	0.21 ± 0.04	0.07 ± 0.01
33	verbenone	1884	0.28 ± 0.17	0.31 ± 0.09	0.11 ± 0.01
34	caryophyllene oxide	1895	0.56 ± 0.27	0.53 ± 0.18	0.13 ± 0.01
35	germacrene-4-ol	2024	2.60 ± 1.45	3.04 ± 0.75	3.26 ± 0.09
36	cubenol	2031	0.51 ± 0.04	0.56 ± 0.08	0.47 ± 0.1
37	spathulenol	2054	1.95 ± 0.44 a	1.93 ± 0.36 a	0.86 ± 0.29 b
38	t-cadinol	2161	1.78 ± 0.19	1.86 ± 0.21	1.57 ± 0.08
39	α-cadinol	2197	6.19 ± 1.35	6.09 ± 0.78	5.17 ± 0.08
	Monoterpene hydrocarbons		57.17 ± 3.04	51.28 ± 5.45	54.23 ± 1.05
	Oxygenated monoterpenes		4.23 ± 2.44	4.36 ± 0.55	3.61 ± 1.76
	Sesquiterpene hydrocarbons		14.48 ± 3.52 c	17.44 ± 2.26 b	21.06 ± 1.89 a
	Oxygenated sesquiterpenes		13.57 ± 2.14	13.95 ± 2.11	11.43 ± 0.22
	Total identified		89.44	87.04	90.32

P < 0.05; *RI on polar column (Zebron ZBFFAP, Phenomenex); n = number of populations.

Discussion

Many wild growing medicinal plants are differentiated when regards their phenotype, i.e. developmental characteristics as well as content and composition of biologically active compounds (Węglarz et al. 2007, Węglarz et al. 2009). Population studies on the chemical diversity were also carried out concerning some coniferous, including *Pinus* species, i.e. *P. nigra* (Šarac et al. 2013), *P. peuce* (Nikoliγ et al. 2014), *P. heldreichii* (Nikolić et al. 2015), *P. caribaea* (Barnola and Cedeno 2000), as well as *P. sylvestris* (Venskutonis et al. 2000, Naydenov et al. 2002, 2005). Scots pine is characterized by a special intraspecific variability caused by high genetic diversity on one side, and significant environmental adaptability on the other (Gawron 2014). On the area of Poland, specific valuable ecotypes of Scots pine were identified (Fonder et al. 2007). Taking into account the differences between these ecotypes concerning some morphological and developmental traits, e.g. tree-stands structure or the rate of growth, it can be assumed that they will also vary in terms of chemical composition.

In the present study, it was observed that the essential oil content in shoot buds and needles increase with increasing latitude. The Polish Forest Growth Regions, which cover the area of our research, differ significantly in terms of the average temperature and annual rainfall, as well as geological and soil conditions. The most northern region (Mazury-Podlasie), so called Polish cold pole, is characterized by the lowest annual temperature and the shortest thermal vegetation period (Zielony and Kliczkowska 2012). Therefore, observed differences in essential oil content in Scots pine buds and needles may be associated with the influence of temperature or day length. In general, results obtained in the present work correspond with data shown by other authors. According to Waliszewska et al. (2005), the content of essential oil in needles is at a level of 0.29-1.09%, Ustun et al. (2006) provides the values from 0.22 to 0.82%, while the results given by Tümen et al. (2010) indicate a content of 0.13%. However, there is no information in literature on total content of essential oil in shoot buds. When regard the composition of shoot bud essential oil, research conducted by Sjödin et al. (2000) and Thoss et al. (2007) showed the domination of δ-3-carene and α-pinene in this raw material, what was confirmed in our study. Given needle essential oil, results obtained in the present study indicate that it contains predominantly α-pinene followed by δ-3-carene, camphene, δ-cadinen and α-cadinol. These data correspond with the results obtained by other authors (Orav et al. 1996, Venskutonis et al. 2000, Judzentiene et al. 2006, etc.). Naydenov et al. (2005) show that the content of α-pinene in essential oil of two-year-old needles collected from different Scots pine populations in the range from 46.83 to 59.27%, while δ-3-carene is at a level of 2.08-5.86%. Venskutonis et al. (2000) identified 70 compounds in needle essential oil, with the clear domination of α-pinene (18.5-33.0%) and δ-3-carene (9.1-24.6%) as well.

Taking into consideration the content of δ-3-carene in pine needle essential oil, two main Scots pine chemotypes are distinguished: in Europe, observed common chemotype with high δ-3-carene content (Orav et al. 1996, Venskutonis et al. 2000, Judzentiene et al. 2006, Kupcinskiene et al. 2008, Judzentiene and Kupcinskiene 2008, Tümen et al. 2010), and a rather rare chemotype characterized by very low content or lack of this substance in the essential oil (Hiltunen et al. 1975, Chalchat et al. 1985, Weissmann and Lange 1990, Semiz et al. 2007). Results obtained by Tobolski and Hanover (1971) indicate that the chemotype of low-δ-3-carene content is characteristic for southern Europe, what could result from the spatial isolation of these areas and evolutionary history of the species. According to Sinclair et al. (1999), the isolated populations of Scots pine, found in southern-western Europe, are considered to be a tertiary relict. Taking into account that in our study δ-3-carene was present in high quantities (8.78 – 9.25%) in all the investigated populations, they can be distinguished as δ-3-carene chemotype, common in central and northern parts of Europe.

In our study, the content of monoterpene hydrocarbons increased with increasing latitude. This tendency, followed by decrease in δ-3-carene, was previously observed by other authors (Muona et al. 1986, Nerg et al. 1994, Manninen et al. 1998, Manninen et al. 2002). According to Yazdani and Nilson (1986), the northernmost populations of Scots pine are characterized by the highest content of limonene in cortical oleoresin. Results obtained in the present study indicate also that the content of sesquiterpene hydrocarbons decrease significantly with increasing latitude. Such phenomenon may be related with the fact that the synthesis of certain terpenes depends on temperatures and light (Lincoln and Langheim 1978, Gleizes et al. 1980). In the experiment conducted by Manninen et al. (1998), the populations of Scots pine from the northern regions of Sweden, with the highest share of monoterpenes in the essential oil, were the least attractive to herbivorous pests, both in terms of feeding on leaves and laying eggs. At once, the populations from the regions with the lowest latitude, poor in monoterpenes – were favoured by these pests. The protective activity of monoterpenes against herbivores was also confirmed by Litvak and Monson (1998). In our study, populations located in the northern part of Poland (Mazury-Podlasie) were characterized by the highest content of monoterpenes as well. It is worth noting that in this area there is the highest frequency and density of self-sown Scots pine trees growing on post-agricultural lands in comparison to other parts of Poland (Buraczyk 2013). This phenomenon could be explained by the above-mentioned resistance to pests as well as with inhibitory effect of monoterpenes on seeds germination of other species growing close to Scots pines (Vokou et al. 2003, Maciąg et al. 2007). Taking into account that monoterpenes such as α-pinene and β-pinene demonstrate strong antimicrobial activity (Krauze-Baranowska et al. 2002, Kohlműnzer 2010), populations with a high content of these compounds in essential oil may also be more interesting source of herbal raw materials as well.

Conclusions

Self-sown Scots pine from the examined Polish Forest Growth Regions differ clearly in respect of the content and composition of essential oils. Populations from the northernmost regions (Mazury–Podlasie) were distinguished by the highest essential oil content and the highest share of α-pinene and β-pinene in essential oil both in shoot buds and needles. Despite the chemical differences, a high content of δ-3-carene in essential oil of all the investigated populations confirm their affiliations to δ-3-carene chemotype. Taking into account the obtained results, further studies on Scots pine population should be undertaken with special respect to the intrapopulation chemical variability of the species. The accumulation of terpenes in Scots pine shoots during their development, from the stage of shoot buds until the full needle formation should be also determined. The results of such studies may be useful to describe the real degree of Scots pine diversity in Poland.

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