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Intraspecific variability of self-sown pine (*Pinus silvestris* L.) occurring in eastern area of Poland

INTRODUCTION

Poland and other Baltic countries seem to be the area where Scots pine finds good environmental growth conditions and creates valuables ecotypes (1). Due to its low habitats requirements and easy adaptation to different soils, this tree occupies over 70% of Polish forests area. It results that self-sown plants of this species can be commonly found on various types of wastelands, often on poor and sandy sites (2). Scots pine is known as a medicinal plant providing important herbal raw materials such as shoot buds (*Pini Gemmae*) and young shoots (*Pini Turiones*). Extracts from pine buds reveal mainly expectorant activity and can be used in respiratory tract diseases treatment while essential oil of pine shoots indicates antibacterial, spasmolytic and warming properties (3, 4). There are many literature data focused on chemical composition of essential oil from pine needles (4, 5, 6, 7) shoots and cones (7) but only few concerning buds (8).

The aim of the present work was to determine intraspecific variability of Scots pine occurring on natural sites in Poland, concerning the content and composition of essential oil in shoot buds.

Photo 1. Scots pine on natural site in Rydzewo

Photo 2. Scots pine on natural site in Istok

MATERIAL AND METHODS

Research covered the eastern region of Poland considered as relatively ecologically clean. In 2012 pine buds were collected from self-sown plants growing wild on 27 natural sites, particularly wastelands or areas near forests, excluded from agricultural use. Geographical location of these sites was determined using GPS (Table 1, Figure 1). The harvest took place in March when buds were still dormant and closed. Collected raw material was dried in 35°C and subjected to chemical analysis. The content of essential oil was evaluated using hydrodestilation in Deryng apparatus, according to Polish Pharmacopeia VIII. Its composition was determined by GC and GC/MS methods. GC analyses were performed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and capillary, polar column HP 20M. The following temperature programme was used: oven temperature isotherm at 60°C for 2 min., then it was programmed from 60°C to 220°C at a rate of 4°C min-1 and held isothermal at 220°C for 5 min. Essential oil compounds identification was done 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 (C7-C30), under the same operating conditions. Retention indices of compounds were also compared with those reported in the literature. The GC/MS analyses were carried out using Shimadzu GC MS QP210S gas chromatograph equipped with Phenomenex Zebron ZBFFAP polar column. The operating conditions were as follows: oven temperature 2 min. isothermal at 60°C, then rising at 4°C/min to 210 °C and held isothermal for 5 min. Injector and detector temperatures: 210 °C and 280 °C, respectively. Ion source temperature -220°C, ionization voltage 70 eV. Mass spectra were scanned in the range 40-500 amu. Essential oil compounds identification was based on comparison of GC retention indices relative to retention times of a series of n-hydrocarbons (C7–C30) with those reported in literature and by comparison of mass spectra from the Mass Spectral Database, as following: NIST08, NIST27, NIST147, Wiley7N2, PAL600K. The percentage composition of the oils was computed by the normalization method from the GC peak areas, without the use of correction factors.

RESULTS

Obtained results show that investigated populations of Scots pine differ both in the content and chemical composition of essential oil in shoot buds. The essential oil content ranged from 0.60 (population Kąty) to 1.87% (population Hajnówka). Based on GC/MS method 31 compounds were identified whereas the predominant was the fraction of monoterpenes. It was characterized by very high percentage of δ-3-carene (from 29.64 population Rydzewo to 52.86% population Biardy), followed by α-pinene (from 7.19 population Stare Lubiejewo to 16.46% population Kałuszyn) and β-pinene (from 3.91 population Biała Podlaska to 14.0% population Istok). Other monoterpenes i.e. limonene and p-cymene-8-ol were also present in high amounts: up to 12.38 and 6.49%, respectively. According to Kupciskiene et al (2008) and Maciąg et al (2007) the dominant fraction in pine needles was found to be monotherpenes, with the major compound α-pinene, followed by δ-3-carene. Sesquiterpenes i.e. β caryophyllene, germakren D, γ and δ cadinene were also present in a considerable amount. The composition of Scots pine essential oil needles could depend on geographical location of pine trees (Maciąg et al 2007). Results obtained by Thoss et al (2007) show the differences in chemical composition of essential oil obtained from one-year old needles in comparison to elder ones. Authors found that the content of δ-3-carene was higher in the essential oil from younger needles than in mature ones. These results were confirmed in the presented study whereas δ-3-carene was the dominant compound in the essential oil of pine buds, which are the earliest stage of pine needles development.

Table 1. Localization of Scots pine natural sites

L.p.	natural site	geographical location N	geographical location E
1.	Trzcianka	52 39.589	21 34.777
2.	Poręba	52 41.236	21 40.832
3.	Stare Lubiejewo	52 51.021	21 52.135
4.	Piski	52 59.782	21 55.459
5.	Stare Rakowo	53 20.376	21 59.510
6.	Piaski	53 14.550	21 52.896
7.	Rydzewo	53 07.892	21 43.126
8.	Kałuszyn	52 12.478	21 49.913
9.	Biardy	52 00.584	22 19.557
10.	Ulan Mały	51 47.613	22 31.411
11.	Rudno	51 46.644	22 53.018
12.	Kożanówka	51 50.451	23 08.593
13.	Koryciny	52 38.447	22 45.637
14.	Skiwy Duże	52 28.695	22 46.969
15.	Słochy	52 23.912	22 47.097
16.	Siemiatycze	52 23.592	22 54.852
17.	Sarnaki	52 18.642	22 54.749
18	Biała Podlaska	52 02.316	23 04.783
19	Świnoroje	52 82.467	23 73.326
20	Istok	52 84.804	23 43.543
21	Hajnówka	52 74.517	23 58.168
22	HutaTurobińska	50 47.950	22 40.950
23	Kąty	50 38.700	22 44.500
24	Panasówka	50 36.483	22 52.533
25	Szozdy	50 35.083	22 56.083
26	Lipowiec	50 37.983	22 50.633
27	Czarnystok	50 39.717	22 52.267

Figure 1. The distribution of investigated populations on area of Poland

Table 2. Total content and composition of essential oils from shoot buds of investigated populations of Scots pine (%)

natural site/populations		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
essential oil content (%)		1.07	0.93	1.07	0.93	1.47	1.07	0.65	0.87	1.67	1.05	0.63	1.09	0.79	1.27	1.40	1.33	1.05	1.20	0.67	1.60	1.87	0.67	0.60	0.67	0.67	0.60	0.93
compound	RI*–Retention Index. HP 20M polar column																											
α thujene	1013	0.21	0.19	0.21	0.30	0.08	0.22	-	0.13	0.32	-	0.24	0.21	0.34	0.38	0.43	0.34	0.29	0.11	0.26	0.35	0.20	0.36	0.33	0.35	0.36	0.28	0.29
α pinene	1028	12.11	14.25	7.19	8.47	13.65	10.70	10.62	16.46	11.47	8.41	11.45	11.89	15.72	13.96	11.48	16.14	14.52	9.34	14.55	12.03	16.43	12.74	10.49	12.91	11.84	12.21	15.37
camphene	1088	0.30	0.30	0.10	0.24	0.38	0.36	0.48	0.54	0.31	0.34	0.44	0.34	0.45	0.52	0.33	0.49	0.43	0.28	0.49	0.35	0.37	0.38	0.31	0.34	0.37	0.34	0.34
β pinene	1113	9.28	4.67	4.52	5.73	6.68	6.65	7.26	10.23	5.99	6.80	5.56	8.61	6.63	8.78	11.43	10.26	5.43	3.91	4.99	14.00	7.36	6.77	7.30	6.17	12.19	8.20	6.54
sabinene	1124	0.74	0.64	0.70	0.70	0.45	0.77	0.46	0.72	0.97	0.57	0.69	0.73	0.62	0.81	0.84	0.89	0.85	0.65	0.80	1.04	0.73	0.81	0.64	0.60	0.68	0.84	0.79
δ-3-carene	1151	44.34	42.46	18.7	45.85	37.97	46.51	29.64	38.06	52.86	31.77	31.77	38.55	36.21	37.86	43.03	43.41	41.09	36.66	36.64	40.12	38.17	45.11	38.72	37.14	37.81	41.49	40.65
β myrcene	1166	1.35	2.67	0.90	1.10	3.88	1.70	2.30	4.86	1.70	1.81	1.57	2.31	3.05	1.65	1.20	1.55	3.31	1.67	1.96	2.00	2.12	1.80	1.10	1.05	2.07	2.08	1.87
trans-3 caren-2-ol	1175	1.66	1.33	1.87	1.94	1.46	1.76	1.58	1.44	1.67	1.32	1.50	1.29	1.47	1.43	1.38	1.18	1.26	1.34	1.24	1.29	0.11	2.03	1.84	2.10	1.37	1.35	1.46
limonene	1203	6.32	12.06	4.49	5.67	7.59	5.56	8.63	4.21	5.11	8.36	6.94	9.56	5.67	9.47	6.20	9.50	7.49	11.40	12.38	4.72	9.76	3.70	5.15	6.56	6.28	4.75	3.79
β phellandrene	1207	1.87	2.69	0.89	1.49	1.78	1.31	1.22	2.00	1.19	1.32	1.59	2.12	1.83	1.78	1.44	2.12	2.71	1.47	2.94	1.45	2.88	1.82	1.15	1.68	2.30	2.21	2.91
ocymene	1265	2.09	2.01	2.82	2.64	2.45	2.47	2.30	2.10	2.20	2.25	2.27	2.00	2.17	1.69	1.71	1.56	1.64	2.27	1.78	1.67	1.26	2.24	2.25	2.09	1.61	1.62	1.69
α terpinolene	1278	0.90	0.68	0.05	0.69	0.25	0.72	0.22	0.39	1.00	0.15	0.37	0.66	0.31	0.89	1.24	1.15	1.24	0.51	0.96	1.10	1.20	1.31	0.98	0.99	1.41	1.72	1.43
limonene oxide	1426	0.36	0.33	0.44	0.44	0.45	0.42	-	0.28	0.31	0.32	0.31	0.30	0.33	0.26	0.25	-	0.26	0.43	0.31	0.23	0.23	-	0.22	0.39	0.26	0.27	0.28
yunipene	1557	-	-	0.20	-	-	-	0.41	0.17	-	0.73	0.14	0.14	0.16	0.12	0.12	-	0.32	1.03	0.59	0.34	0.38	0.48	0.40	0.43	0.49	0.45	0.27
bornyl acetate	1576	0.37	0.55	1.46	0.65	0.89	0.40	0.64	0.88	0.39	1.06	0.81	0.60	0.66	0.71	0.33	0.49	0.73	1.28	0.95	0.48	0.27	-	0.62	0.49	0.57	0.44	0.31
β caryophyllene	1593	-	0.23	-	0.42	0.46	0.74	1.74	-	-	-	-	-	-	1.19	1.30	1.14	1.60	2.20	1.68	1.16	1.29	1.78	1.63	1.53	1.31	1.48	1.33
terpinen-4-ol	1596	1.65	1.79	1.63	1.95	2.23	1.70	2.20	1.57	1.47	1.81	1.76	1.54	1.54	0.46	0.25	-	0.26	0.37	0.43	0.28	0.31	-	0.34	0.41	0.42	0.32	0.32
myrtenal	1608	0.35	0.31	0.44	0.30	0.43	0.34	0.69	0.35	-	0.44	0.31	0.50	0.35	0.82	0.75	0.65	0.61	0.92	0.67	0.80	0.65	0.91	0.96	0.90	1.10	0.79	0.81
pinocarveole	1635	0.96	0.65	0.08	0.75	1.09	0.89	1.68	0.92	0.58	1.21	0.80	0.93	0.88	0.38	0.34	-	0.42	0.58	0.38	0.29	0.32	0.50	0.38	0.63	0.42	0.40	0.41
isoborneole	1657	0.37	0.36	0.38	0.42	0.29	0.33	0.62	0.35	0.24	0.60	0.45	0.43	0.47	1.06	1.00	0.83	0.94	1.53	1.03	0.97	1.08	1.15	1.54	1.55	1.25	1.19	1.12
α-terpineol acetate	1677	1.39	1.02	1.65	1.56	1.60	1.54	2.32	1.14	0.94	1.68	1.44	1.36	1.39	0.62	0.73	0.59	0.80	1.22	0.88	0.54	0.78	1.01	1.16	1.02	0.84	0.85	0.82
α-terpineol	1681	0.99	0.54	1.37	1.56	1.14	1.50	2.10	0.78	0.72	1.28	0.96	0.78	1.02	2.66	2.35	1.88	2.11	1.28	1.66	2.04	1.87	3.82	2.48	3.32	2.63	2.54	2.61
α phellandren 8 ol	1696	1.45	0.90	0.14	1.77	0.91	1.41	0.98	0.72	1.50	0.84	0.98	1.24	0.97	1.22	1.19	0.94	1.02	0.29	0.74	0.99	0.94	1.99	0.37	1.75	1.46	1.31	1.49
carvone	1711	0.37	0.39	0.52	0.52	0.53	0.32	0.70	0.24	0.40	0.69	0.45	0.45	0.50	0.36	0.33	-	0.33	0.79	0.44	0.31	0.29	0.56	0.59	0.52	0.37	0.35	0.39
germakren D	1725	0.50	0.39	0.92	0.61	0.52	0.55	0.72	0.40	0.40	0.85	0.61	0.39	0.47	0.40	0.42	0.32	0.27	0.45	0.31	0.95	0.30	0.44	0.19	0.46	0.65	0.43	0.41
γ cadinene	1793	0.49	0.29	0.40	0.35	0.53	0.43	0.91	0.49	0.29	0.64	0.39	0.46	0.42	0.47	0.30	0.30	0.38	0.88	0.59	0.29	0.40	-	0.40	0.43	0.38	0.32	0.31
carveol	1810	0.31	0.49	0.44	0.31	0.44	0.33	0.91	0.23	0.29	0.65	0.44	0.51	0.44	2.86	2.61	2.04	2.37	5.30	2.67	2.45	2.32	4.10	4.85	3.92	2.83	2.40	2.87
p cymen-8-ol	1819	4.13	3.06	6.40	5.35	4.87	4.87	6.49	3.20	3.53	5.82	4.72	3.51	4.03	1.63	1.60	1.30	1.56	3.17	1.62	1.61	1.27	2.13	2.48	1.89	1.54	1.37	1.60
geraniole	1826	2.41	1.76	3.48	2.92	2.65	2.78	3.45	1.85	2.24	3.04	2.45	2.14	2.15	0.13	0.10	-	0.10	0.43	0.16	0.13		-	0.28	0.14	0.15	0.09	0.11
verbenone	1884	0.30	0.29	1.17	0.36	0.49	0.35	0.49	0.34	0.28	0.68	0.47	0.28	0.36	0.15	0.18	-	0.32	0.47	0.34	0.12	0.21	-	0.28	0.34	0.21	0.28	0.12

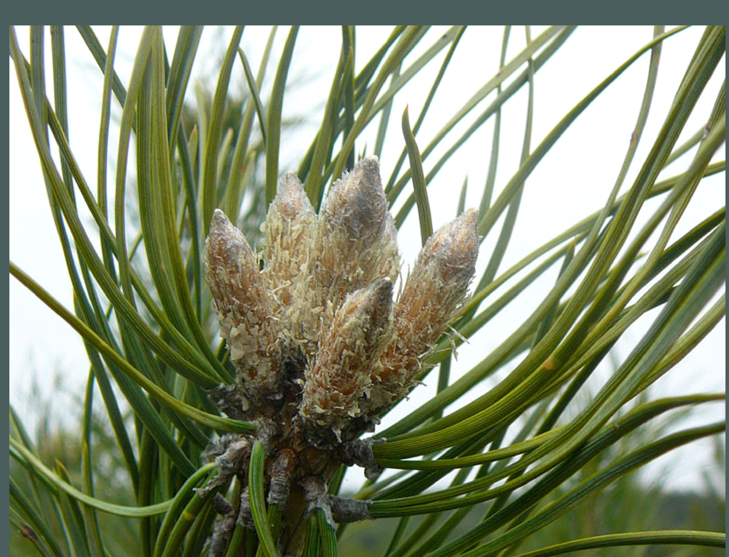

Photo 3. Scots pine shoot buds

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