Comparative analysis of monoterpene composition in four pine species with regard to suitability for needle consumer *Diprion pini* L.

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In the needles of 4 pine species (*Pinus. sylvestris, P. nigra, P. banksiana* and *P. strobus*) 15 volatile monoterpenes were identified by GC-MS analysis using synthetic standards. Interspecific differences were established in the total amount of monoterpenes and their proportion. The principal component analysis (PCA) of the compounds revealed two clusters similar in the composition of monoterpenes. The clusters corresponded well to the oviposition preference of *Diprion pini,* which is among the main folivorous insects causing defoliation of *Pinus* spp. in Europe. One monoterpene cluster included *P. banksiana* and *P. strobus* (not suitable for *D. pini* oviposition, Barre et al., 2002) and another one included *P. sylvestris* and *P. nigra* (suitable for *D. pini* oviposition, Barre et al., 2002). The main compounds that stipulated cluster divergence were limonene, β-pinene and myrcene. Relative concentrations of these metabolites were significantly higher in *P. banksiana* and *P. strobus*. Further studies on the perception of monoterpenes and their behavioural effect on *D. pini* are in progress.

Key words: secondary metabolites, PCA, limonene, β-pinene, myrcene, chemoecology, plant-insect interaction

INTRODUCTION

Plants produce over 15000 terpenes (Langeneim, 1994). They function either as protection agents or / and signals (Peñuelas, Llusià, 2003). Coniferous terpene compounds are extremely important, because they act either as repellents against potential pests (especially phytophagous insects) or as toxic substances both for pests and pathogens (Delorme, Lieutier, 1990; Raffa, Smalley, 1995; Klepzig et al., 1996). Because of these substances, conifers defend themselves both directly and indirectly. E. g. Scots pine (*Pinus sylvestris* L.) defends indirectly against attacks of the pine sawfly *Diprion pini* L. (Hymenoptara: Diprionidae) at the egg laying stage. Following pine sawfly oviposition, the pine emits volatile terpenoids attractive for egg parasitoids of the phytophagous insect (Schroder, 2007). The plant which reacts to damage (or even

potential damage) modifies emission of secondary metabolites either quantitatively or qualitatively (e. g., Boland et al., 1999; Dicke, 1999; Parʼe et al., 1999).

A pine defoliator pine sawfly *D. pini* is a pest of great economic importance. Massive outbreaks of the species are very dangerous not only because of very high local damage, but also of huge damaged forest plots (Somviele, 2004). Gravid females of *D. pini* are capable to choose a pine tree suitable for egg laying, and the most vulnerable is the Scots pine (*Pinus sylvestris* L.), while the Eastern white pine (*P. Strobes* L.) or Banksy pine (*P. banksiana* Lambert) are chosen very rarely. The European black pine (*P. nigra* J. F. Arnold) can also be chosen for egg laying though the development of pine sawfly larvae is slowed down (Barre et al., 2003).

The aims of the present study are: (1) to determine the composition of 15 volatile monoterpenes in the needles of 4 pine species which differ

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in suitability for egg laying by pine sawfly females; (2) by means of the principal component analysis (PCA) to define monoterpenes patterns of each pine species; and (3) to make a comparative analysis of pine monoterpene composition and egg laying preference of a phytophagous insect *D. pini*.

MATERIALS AND METHODS

One- and two-year-old needles were collected from pines *Pinus sylvestris*, *P. strobus*, *P. nigra* and *P. banksiana* (6 samples from 3 trees of each species) growing in Vilnius and Klaipėda botanical gardens (Lithuania). The monoterpene composition in pine needles differs seasonally (Petrakis et al., 2001). To diminish monoterpene seasonal variation discrepancies, the samples were taken in October 2009 and July 2010. The samples were taken from a particular pine tree and from the same branch to avoid polymorphic differences. After collection, the samples were closed in plastic bags and transported into the laboratory where they were kept frozen (–16 °C) until analysis.

After the needles (0.5 g) were cut and placed in the vials, 2 ml hexane spiked with 1 µg/ml of internal standard (tetradecane) was added into each vial. Those were capped and stored in the dark at 4 °C temperature for 48 h to extract monoterpenes. Each solution was filtered through 150–200 mg of silica gel. The extracts were stored at –18 °C before the analysis.

To determine dry matter of extracted needles, the vials were uncapped and left to evaporate and the needles were dried for 48 h at 50 °C in the vials. The needles were removed and weighed to 0.01 mg accuracy.

GC-analysis. Monoterpene composition was evaluated by GC analysis. The PerkineElmer GC model Clarus 500 equipped with a flame ionization detector (FID) and polar capillary column DB-Wax (30 m; 0.25-mm i. d., 0.25-µm film-thickness, Agilent Technologies, USA) was used. The temperature program started at 40 °C (held for 1 min), then raised by 5 °C min^{-1} to 200 °C, then by 10 °C min^{-1} to 240 °C and held at 240 °C for 11 min. The injector temperature was 240 °C, the detector temperature was 240 °C. Hydrogen was used as carrier gas at a flow of 2.5 ml min^{-1}. One µl of the extract was injected for analysis.

GC-MS analysis. The extracts were analysed by a Shimadzu GC 2010 connected to a MS-QP 2010 MS. A Stabilwax fused silica capillary column (30 m; 0.25-mm i. d., 0.25-µm film-thickness, Restek, USA) was used and the temperature program was the following: isothermal at 40 °C for 2 min, afterwards increased to 200 °C at 5 °C min^{-1}, then to 240 °C at 10 °C min^{-1}, and finally isothermal at 240 °C for 20 min. The injector temperature was 240 °C. Helium was used as carrier gas (inlet pressure 67.7 kPa). Electron-impact (EI) spectra were acquired at the electron energy of 70 eV, the ion-trap source temperature was 250 °C. Spectra were continuously scanned from mass 33 to 500 at 0.5 scan/sec. One µl of hexane extract was injected into GC-MS manually by using a 10 µl syringe. The monoterpenes were identified by comparing retention times and mass spectra with those of available authenticated standards, or by comparing retention indexes (RIs) and mass spectra with those from the reference library of NIST08 (National Institute of Standards and Technology). Synthetic standards of α-pinene, β-pinene, 3-carene were obtained from Roth, terpinene, (*Z*)-β-ocimene from Aldrich, myrcene, limonene from Fluka, and camphene from Supelco. The absolute concentrations of terpenes were calculated relative to the internal standard and expressed as µg g^{-1} dry wt., as well as tetradecane equivalents.

Statistical analysis. The data of all 15 volatile compounds were used for quantitative analysis. Relative amounts were evaluated by multivariate data analysis using the software Statistica 6.0. The data were subjected to the principal components analysis (PCA). The PCA was conducted in order to extract and display the systematic variation in the multivariate data set consisting either of the total amount or proportions of 15 compounds (variables) obtained from 24 replicates (observations). Raw data (integrated peak area in each chromatogram) were normalized, i. e. peak areas of 15 compounds were summed to 100 and the percentage of each variable was calculated. One-way ANOVA and Ficher LSD ($P < 0.05$) were performed to compare differences of compounds among pine species.

RESULTS

Composition and concentrations of monoterpenes in pine needles

By means of GC and GC-MS analysis of extracts from needles of four pine species (*P. sylvestris*, *P. nigra*, *P. banksiana*, *P. strobus*), 15 volatile monoterpenes were identified (Table). All 15 monoterpenes were present in all 24 samples collected from 4 pine species. The monoterpenes are common not only for pines, but also for many other coniferous species (Macchioni et al., 2003). GC/FID chromatogram examples of needle extracts of 4 pine species with marked monoterpenes are presented in Fig. 1. No qualitative differences were revealed in the monoterpene composition within needles of all four pine species. Monoterpenes differed in absolute as well as relative concentrations. The highest significant absolute amount of monoterpenes (4170.46 ± 221.98 µgg⁻¹) was registered in the needles of *P. sylvestris*, lower amounts were determined for *P. nigra* and *P. strobus* (3198.03 ± 274.79 and 2187.31 ± 206.42 µgg⁻¹, respectively); and the lowest amount was found in *P. banksiana* (1136.33 ± 240.96 µgg⁻¹) needles (Table).

Interspecific comparison of monoterpene content revealed the highest absolute amount of 3-carene (1585.80 ± 192.70 µgg⁻¹), γ-terpinene (12.44 ± 1.55 µgg⁻¹), terpinolene (114.44 ± 15.06 µgg⁻¹) and sabinene (27.81 ± 4.78 µgg⁻¹) in the needles of the Scots pine. The needles of the species contained the lowest amount of β-pinene (68.24 ± 11.16 µgg⁻¹). Most of α-pinene (2761.72 ± 254.07 µgg⁻¹) and least of both 3-carene (6.99 ± 0.73) and camphene (65.69 ± 6.43 µgg⁻¹) were determined in the European black pine. The astern white pine was distinguished by higher absolute amount both of β-pinene (327.83 ± 26.23 µgg⁻¹) and β-phellandrene (120.32 ± 13.92 µgg⁻¹). Eastern white pine needles contained the least amount of α-pinene (596.96 ± 158.35 µgg⁻¹). In the needles of *P. sylvestris* and *P. nigra*, the highest amount of E-β-ocimene was present (44.29 ± 7.94 µgg⁻¹ and 46.55 ± 6.60 µgg⁻¹, respectively). *P. strobus* and *P. banksiana* needles contained an extremely low amount of the latter monoterpene (up to 3 µgg⁻¹). Among the monoterpenes analysed, the needles of all 4 investigated pine species contained the lowest amount of *p*-cimene ir γ-terpinene (Table).

Relative amounts of monoterpenes in pine needles

Analysis of relative concentrations of monoterpenes in pine needles revealed a different composition of the compounds in each pine species.

Relative concentration of α-pinene was the highest in the needles of all analysed pine species.

P. sylvestris contained the highest proportion both of α-pinene and 3-carene (35.93 ± 10.87%) and terpinolene (2.28 ± 0.95%) compared to the other pine species. A very high relative amount of α-pinene and 3-carene (both compounds made 84.3% of all monoterpenes) was characteristic of this species.

In addition to a high amount of α-pinene, *P. nigra* contained β-pinene (4.54 ± 1.56%) and camphene (2.33 ± 0.6%).

Along with the highest amount of α-pinene, *P. strobus* needles were distinguished by high amounts of β-pinene (15.07 ± 1.84%), α-phelandrene (2.97 ± 0.53%) and β-phelandrene (5.38 ± 0.77%).

Among all the investigated pine species, *P. banksiana* contained especially high proportions both of α-pinene (48.14 ± 1.5%) and camphene (16.87 ± 0.74%). According to monoterpene composition, this pine species was to some extent similar to *P. strobus*, as characteristic of both was the higher relative amount of β-pinene (14.97 ± 4.16%), limonene (2.41 ± 0.86%) and myrcene (5.29 ± 1.32%) compared to the rest of the species.

Basing on interspecific comparison of relative amounts of monoterpenes in the needles, one can conclude that characteristic of all the species was high proportion of α-pinene (more than 50%) and low proportion of *p*-cimene (0.45% and less).

Principal components analysis

The PCA of absolute concentrations of 15 monoterpenes did not reveal any pattern of clustering by the species; however, analysis of relative amounts of the same monoterpenes grouped pine species into 2 clusters.

The PCA plot of relative amounts of monoterpenes in the needles of four pine species is presented in Fig. 2a. The first two principal factors
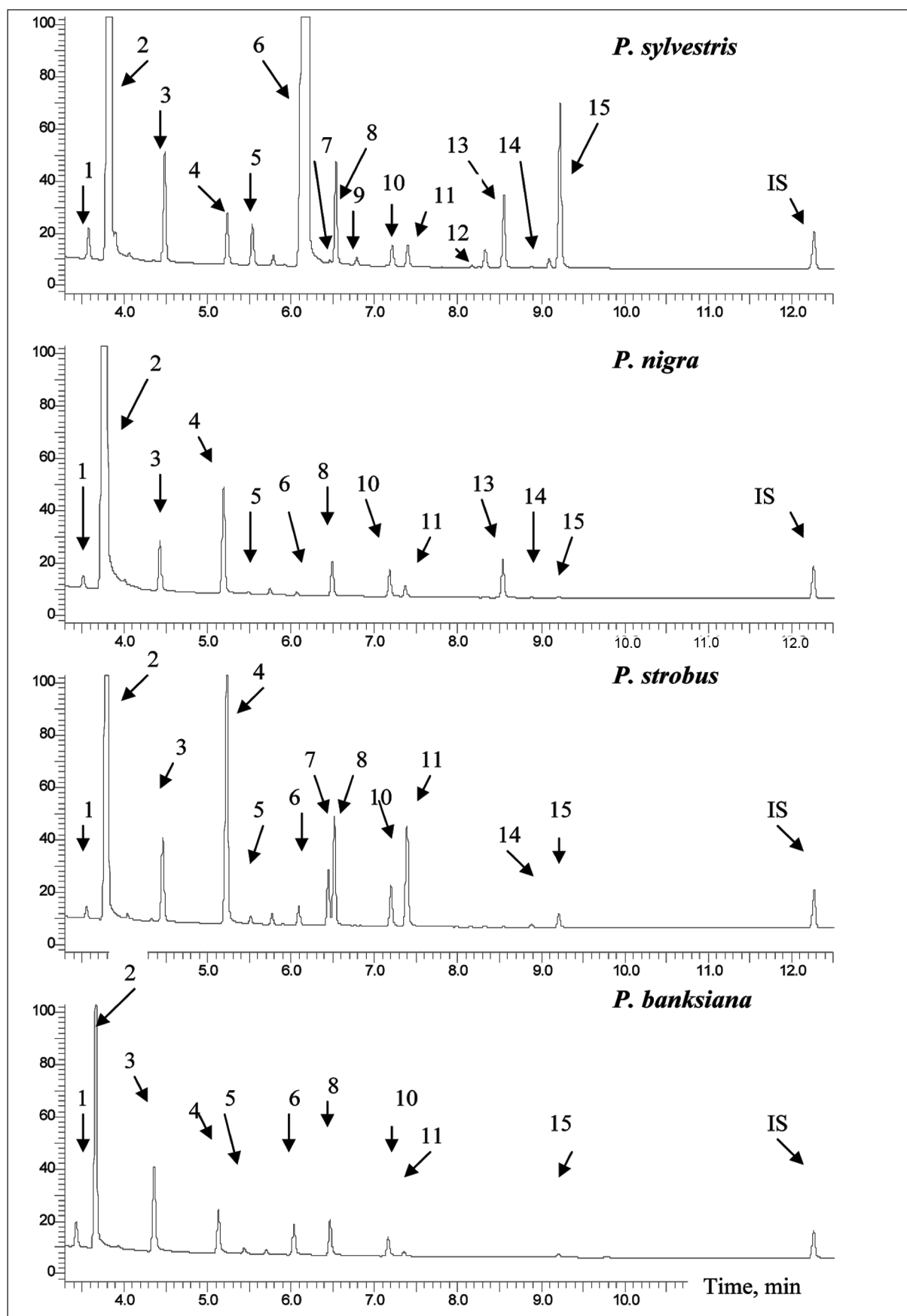
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Fig. 1. GC/FID cromatograms of monoterpenes in the needles of four pine species (*P. sylvestris*, *P. nigra, P. strobus, P. banksiana*). For numbers indicating monoterpenes see Table

Table. Absolute amounts of monoterpenes (μgg⁻¹ dry weight equivalent to tetradecane; percentage calculated from GC chromatogram peaks) in the needles of four pine species (*P. sylvestris, P. nigra, P. strobus, P. banksiana*)

No	RI[a]	Compound	*Pinus sylvestris*		*Pinus nigra*		*Pinus strobus*		*Pinus banksiana*	
			Mean ± SE μgg⁻¹ dry wt (N = 13)	Mean ± SE % (N = 15)	Mean ± SE μgg⁻¹ dry wt (N = 16)	Mean ± SE % (N = 17)	Mean ± SE μgg⁻¹ dry wt (N = 13)	Mean ± SE % (N = 15)	Mean ± SE μgg⁻¹ dry wt (N = 12)	Mean ± SE % (N = 13)
1	1006	tricyclene	39.54 ± 7.26a	1.10 ± 0.47	17.67 ± 1.65b	0.61 ± 0.16	15.43 ± 1.78b	0.68 ± 0.06	41.94 ± 5.09a	4.55 ± 0.1
2	1024	α-pinene	1982.67 ± 168.28b	48.37 ± 11.11	2761.72 ± 254.07a	85.72 ± 2.65	1238.03 ± 111.25c	55.15 ± 1.8	596.96 ± 158.35d	48.14 ± 1.5
3	1060	camphene	157.79 ± 31.90a	4.63 ± 1.98	65.69 ± 6.43b	2.33 ± 0.6	128.19 ± 16.75a	5.54 ± 0.65	146.20 ± 20.62a	16.87 ± 0.74
4	1101	β-pinene	68.24 ± 11.16a	1.94 ± 0.71	137.50 ± 14.28b	4.54 ± 1.56	327.83 ± 26.23c	15.07 ± 1.84	127.02 ± 16.10b	14.97 ± 4.16
5	1115	sabinene	27.81 ± 4.78a	0.73 ± 0.27	11.65 ± 2.60b	0.37 ± 0.16	11.60 ± 1.60b	0.50 ± 0.06	9.46 ± 1.80b	0.91 ± 0.15
6	1144	3-carene	1585.80 ± 192.70a	35.93 ± 10.87	6.99 ± 0.73c	0.21 ± 0.05	147.77 ± 33.87b	6.05 ± 2.53	93.82 ± 48.24b	4.28 ± 2.13
7	1157	α-phellandrene	1.28 ± 0.30b	0.02 ± 0.01	0.74 ± 0.15b	0.02 ± 0.01	66.97 ± 8.80a	2.97 ± 0.53	1.01 ± 0.79b	0.04 ± 0.02
8	1160	myrcene	83.40 ± 6.40b	2.21 ± 0.17	43.80 ± 3.90c	1.47 ± 0.17	115.13 ± 14.25a	5.21 ± 0.95	51.72 ± 10.30c	5.29 ± 1.32
9	1174	α-terpinene	4.58 ± 0.63a	0.10 ± 0.04	1.20 ± 0.23b	0.05 ± 0.02	0.73 ± 0.15b	0.03 ± 0.00	0.67 ± 0.34b	0.04 ± 0.01
10	1191	limonene	24.25 ± 2.20a	0.53 ± 0.08	46.27 ± 5.19b	1.48 ± 0.19	49.34 ± 5.23b	2.19 ± 0.22	27.51 ± 5.58a	2.41 ± .086
11	1203	β-phellandrene	18.64 ± 1.25b	0.44 ± 0.08	20.73 ± 2.80b	0.62 ± 0.1	120.32 ± 13.92a	5.38 ± 0.77	21.25 ± 6.38b	1.9 ± 0.04
12	1238	γ-terpinene	12.44 ± 1.55a	0.28 ± 0.09	1.45 ± 0.31b	0.04 ± 0.02	1.62 ± 0.28b	0.07 ± 0.02	1.10 ± 0.33b	0.08 ± 0.02
13	1247	E-β-ocimene	44.29 ± 7.94a	0.92 ± 0.50	46.55 ± 6.60a	1.57 ± 0.44	0.36 ± 0.05b	0.02 ± 0.00	2.78 ± 2.38b	0.07 ± 0.01
14	1261	p-cymene	1.02 ± 0.12a	0.45 ± 0.98	0.57 ± 0.07a	0.02 ± 0.00	0.36 ± 0.05b	0.07 ± 0.02	0.82 ± 0.19b	0.06 ± 0.01
15	1275	terpinolene	114.44 ± 15.06a	2.28 ± 0.95	31.51 ± 7.96b	0.94 ± 0.24	25.21 ± 3.69b	1.09 ± 0.16	9.64 ± 5.58b	0.38 ± 0.12
		Total	4170.46 ± 221.98a		3198.03 ± 274.79b		2187.31 ± 206.42c		1136.33 ± 240.96d	

of the components explained 63.56% of monoterpene data variation. Monoterpene distribution in the Scots pine and European black pine was very similar and the values of 15 monoterpenes overlapped. Similar clustering was observed in the monoterpenes of the Eastern white pine and Banksy pine (Fig. 2a). Thus, four pine species were clearly separated into two groups based on the composition of relative amounts of monoterpenes in the needles of these species.
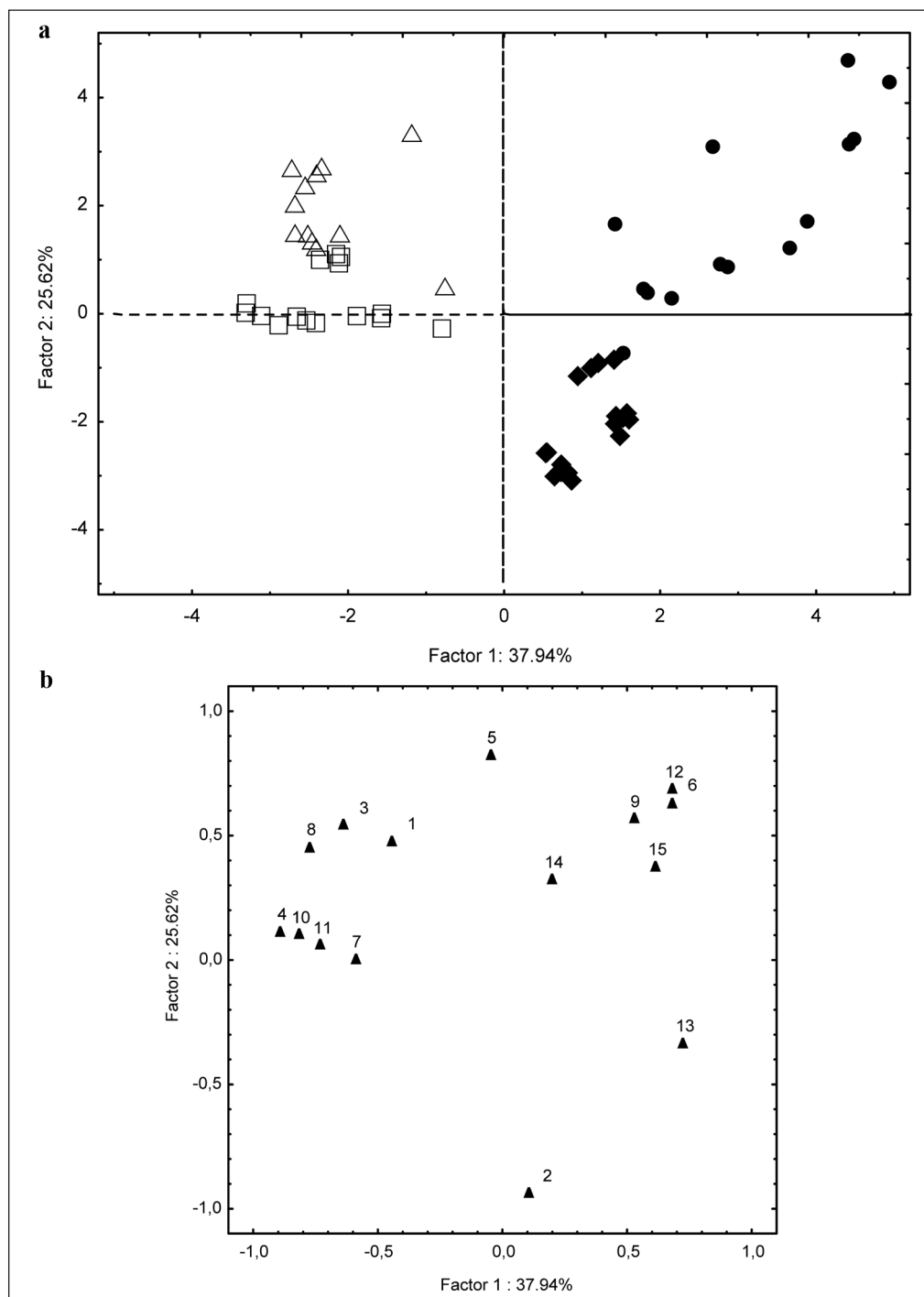

Fig. 2. PCA of 15 monoterpenes in the needles of 4 pine species: (a) value graph, and (b) distribution of the influence of separate monoterpenes. Black circle – *P. sylvestris*, black diamond – *P. nigra*, white triangle – *P. banksiana*, white square – *P. strobus*

The PCA results of the distribution values of individual monoterpene impact are presented in Fig. 2b. The monoterpenes that influenced distribution by the first factor (see Fig. 2a) to the greatest extent (i. e. grouping *P. sylvestris* and *P. nigra* as well as grouping *P. banksiana* and *P. strobus*) were β-pinene, limonene, β-phelandrene, myrcene, γ-terpinene and α-phelandrene. The amounts of β-pinene, limonene and myrcene were significantly higher in *P. banksiana* and *P. strobus* needles (Fig. 3). The distribution of monoterpene composition by the second factor was determined by α-pinene, sabinene and γ-terpinene. A higher proportion of α-pinene determined the vertical secession of *P. nigra* template. In Fig. 2a, *P. sylvestris* template was the widest in PCA graph because of higher variation of α-pinene and 3-carene. The monoterpenes that influenced distribution of values (see Fig. 2a) to the lowest extent were *p*-cimene and tricyclene.

DISSCUSION

The differences revealed in absolute and relative monoterpene concentrations within needles of 4 pine species were quantitative only. This corresponds to the data on volatile emissions in some pine species presented by Pureswaram (2004), Faldt (2001), Mumm (2004), Silvestrini (2004), Dormont (1998), and Tammela (2003).

The absolute amount of α-pinene is well known to be the highest among monoterpenes in various *Pinus* species. The highest absolute amount of both α-pinene and 3-carene revealed in the needles of *P. sylvestris* corresponds to the data published by Corkill (1988) and Raisanen (2009). It should be noted that pines containing a higher amount of α-pinene simultaneously contained a lower amount of 3-carene, and vice versa.

The relative amounts of monoterpenes in the European black pine we recorded were similar to those reported by other authors (Mumm et al., 2004) though our results showed a lower relative amount of β-phelandrene. The highest relative amounts of limonene, β-pinene and myrcene we recorded was in *P. banksiana* and *P. strobus*. The presence of a relatively high content of these monoterpenes in *P. strobus* was noted by other authors as well (Bridgen et al., 1979; Klepzig et al., 1996). A comparative analysis of monoterpene composition in the needles of *P. sylvestris*, *P. nigra* and *P. pinea* demonstrated that the main monoterpenes distinguishing these species were limonene and myrcene (Martini et al., 2010). According to our data, not only limonene and myrcene, but also relative amounts of β-pinene made a reliable difference between the needles of 4 investigated pine species (Fig. 3). According to Petrakis (2004), such result was determined by myrcene and β-pinene.

The proportions of monoterpenes differed depending on the pine species. In our opinion, this is important for plant-insect interactions. The absolute concentration of secondary metabolites emitted by the pine trees could not be the most important for coniferous pests while searching for a tree suitable for egg laying or feeding, because the absolute concentration differs depending on the distance to the emission source, thus cannot provide information on host plant species. As to the proportion of monoterpenes, it remains stable enough within the trail of volatile substances. It was assumed that the pine sawfly chooses the host plant based on the proportion of volatiles, and even small changes in proportions can determine whether the plant will be attractive to the insects or not. Namely, relative changes in infochemical substances mostly determine relationships between phytophagous insects and their host plants (Beyaert et al., 2010).

The PCA distinguished the patterns of relative amounts of monoterpenes only.

Based on the relative amounts of 15 monoterpenes, the PCA grouped 4 pine species into two clusters: Banksy pine and Eastern white pine forming one cluster, and the European black pine and Scots pine another cluster (see Fig. 2a). According to the phylogenetic data of *Pinus* family by Gernandt et al. (2005), *P. sylvestris* and *P. nigra* are phylogenetically closely related species, both belonging to the same section and subsection. *P. banksiana* is a more phylogenetically distant species and belongs to a different *Trifoliae* section. The most phylogenetically distant species, compared to the other three ones that we used in our research, is *P. strobus*, which belongs to section *Quinquefoliae*. The data of the PCA of needle monoteropenes correlates well enough with phylogenetic data.

The results obtained by Barre et al. (2002) demonstrated that pine sawfly females do not choose among pine species accidentally for egg laying.
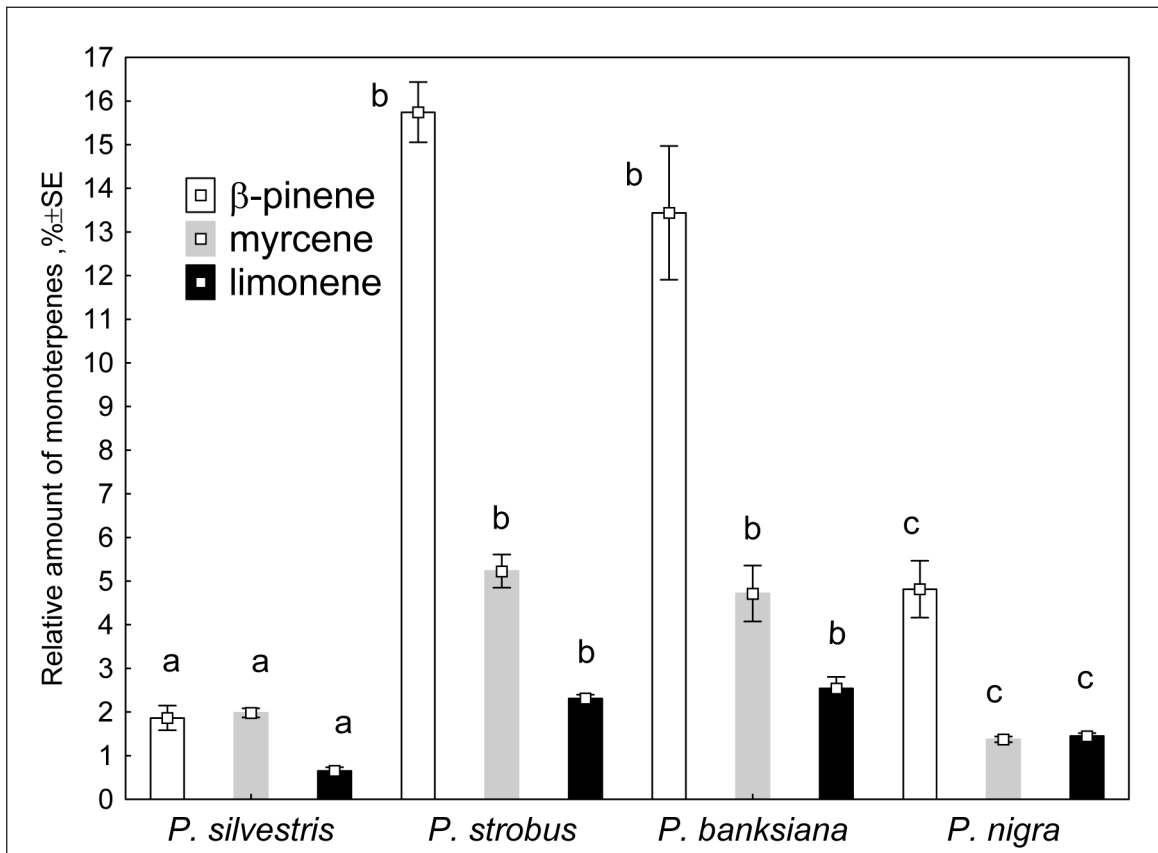
Fig. 3. Relative amounts of β-pinene, myrcene and limonene (average, % ± SE) in the nee-
dles of 4 pine species (*P. sylvestris*, *P. strobus*, *P. banksia*na and *P. nigra*)

D. pini females prefer *P. sylvestris* and *P. nigra* and not *P. strobus* or *P. banksiana*. The larvae survival rate was higher in *P. sylvestris* and *P. nigra*. This corresponds to the PCA clustering of pine species by the monoterpene composition of the needles. The difference between pine species chosen and not chosen by the pine sawfly was determined by the first principal factor of the PCA. Monoterpenes that determine the first principal factor (and possibly influence which species should be chosen for laying eggs by the sawfly) were β-phelandrene, limonene, β-pinene, myrcene, γ-terpinene and α-phelandrene. Relative amounts of β-phelandrene, γ-terpinene and α-phelandrene were statistically higher in *P. strobus*. Relative amounts of the aforementioned monoter-penes did not differ in *P. banksiana* when compared to *P. sylvestris* and *P. nigra*. Thus, β-pinene, limonene and myrcene should be attributed to the main monoterpenes that most probably determine differences between suitable and not suitable trees for *D. pini* oviposition.

We stipulate that the choice by pine sawfly females to lay eggs is determined by differences between relative monoterpene concentrations.

Hilker and Weitzel (1991) stated that *D. pini* females were repelled by 3-carene, α-pinene and β-pinene. The effect of β-pinene is in accordance with our data, as high relative amount of β-pinene was characteristic of those pine species that were not preferred by *D. pini* females. The data obtained later did not demonstrate any influence of 3-care-ne on tree choice by the pine sawfly (Lyytikäinen, 2003). Our PCA also indicated that 3-carene was not among the main monoterpenes for clustering pine species.

Still, there is no reliable data on the role of mo-noterpenes in the attraction or repulsion of *D. pini* females; however, analysis of their role seems to be prospective considering the data on the role of β-pinene, limonene and myrcene as attractants or repellents for closely related sawfly species. E. g., the pine sawfly *Neodiprion sertifer* is attracted to

limonene (at higher concentrations limonene becomes repellent) (Martini et al., 2010). When higher amounts of limonene and β-pinene are emitted following methyl jasmonate (MeJA) application (Zhao, 2010; Heijari et al., 2008), protective responses of conifers commence against phytophagous insects, including *D. pini* (Martin et al., 2003; Heijari et al., 2005). According to our data (Fig. 3), higher relative amounts of limonene were contained in those pine species which were not preferred by *D. pini* females for oviposition.

β-pinene is known as an attractant for some *D. pini* parasitoids (Hymenoptera: Braconidae) (Carrasco, 2005). According to our data (see Fig. 3) a relatively high amount of β-pinene was characteristic of *P. banksiana* and *P. strobus*, i. e. the pine species that are not attractive for egg laying by *D. pini* females. This could be an extra reason for *D. pini* females not to choose particular pine species.

In conclusion, we state that relative amounts of monoterpenes suitable for *D. pini* females to lay eggs differ between 4 pine species (*P. sylvestris*, *P. nigra*, *P. banksiana* and *P. strobus*). β-pinene, myrcene and limonene are suggested to be tested as potential compounds related to host plant choice by the pine sawfly, *D. pini*, gravid females.

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MONOTERPENŲ SUDĖTIES KETURIŲ RŪŠIŲ PUŠŲ SPYGLIUOSE LYGINAMOJI ANALIZĖ ATSIŽVELGIANT Į JŲ TINKAMUMĄ VARTOTOJUI *DIPRION PINI* L.

Santrauka

Keturių rūšių pušų (*Pinus sylvestris*, *P. nigra*, *P. banksiana* ir *P. strobus*) spygliuose, taikant dujų chromatografijos ir masių spektrometrijos metodus bei sintetinių junginių standartus, identifikuota 15 monoterpenų. Nustatyti tarprūšiniai skirtumai tiek suminiuose monoterpenų kiekiuose, tiek jų proporcijose. Pagrindinių komponentų analizė rodo, jog susidaro du klasteriai, panašūs pagal monoterpenų sudėtį. Šie klasteriai atitinka pjūklelio *Diprion pini*, kuris Europos pušynuose yra vienas labiausiai spyglius graužiančių kenkėjų, kiaušinėlių dėjimo preferenciją. Vieną klasterį sudarė *P. banksiana* ir *P. strobus* (*D. pini* kiaušinėliams dėti netinkamos rūšys, Barre et al., 2002) o kitą – *P. sylvestris* ir *P. nigra* (*D. pini* kiaušinėliams dėti tinkamos rūšys, Barre et al., 2002). Svarbiausi junginiai, lemiantys klasterių išsiskyrimą, yra limonenas, β-pinenas ir mircenas. Šių antrinių metabolitų santykinių kiekių yra kur kas daugiau *P. banksiana* ir *P. stropus* pušyse. Numatoma atlikti monoterpenų užuodžiamumo ir šių junginių poveikio pjūklelio *D. pini* elgsenai tyrimus.

Raktažodžiai: antriniai metabolitai, PCA analizės, limonenas, β-pinenas, mircenas, chemoekologija, augalų ir vabzdžių sąveika