Chem. Anal. (Warsaw), **51**, 551 (2006)

Application of Multivariate Statistical Analysis to Biological Data. Variations of Monoterpene Content in Fresh Needles of *Picea abies (L.) Karst*

by Valerie Helánová[1], Iva Chvílíčková[1], Milena Martinková[2], Milan Meloun[3] and Vlastimil Kubáň[1]*

[1] Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry, Zemědělská 1, CZ-613 00 Brno, Czech Republic
[2] Department of Forest Botany, Mendel University of Agriculture and Forestry, Zemědělská 3, CZ-613 00 Brno, Czech Republic
[3] Department of Analytical Chemistry, University of Pardubice, Čs. legií 653, CZ-532 10 Pardubice, Czech Republic

Keywords: Principal component analysis; Correlation analysis; Cluster analysis; ANOVA; Needle age classes; Terpenes; Norway spruce; *Picea abies (L.) Karst*

Gas chromatographic–mass spectrometric (GC–MS) analysis using an HP–INNOWax capillary column (30 m × 0.25 mm × 0.5 μm film of polyethylene glycol) has been used for determination of individual monoterpenes in fresh coniferous needles of *Picea abies (L.) Karst*. Concentration and composition pattern of monoterpenes were investigated with respect to the year of differentiation of needles (age of needles) and characteristics of shoots (length and total dry matter weight, DMW). Principal component analysis, analysis of variance, and cluster analysis allowed one to specify the content and composition pattern of essential oils and to find interrelations (also the latent ones) between year classes of needles.

Opracowano chromatograficzną metodę (chromatografia gazowa sprzężona z detektorem masowym, GC–MS), stosując kolumnę kapilarną HP–INNOWax (30 m × 0,25 mm × × 0,5 μm film glikolu polietylenowego), do oznaczania pojedynczych monoterpenów w świeżych igłach świerku pospolitego, *Picea abies (L.) Karst*. Określano stężenie i skład monoterpenów w zależności od wieku igieł i charakterystyki parametrów wzrostu pędów (długość i całkowity ciężar suchej masy, DMW). Analiza głównego składnika, analiza wariancji i analiza grup pozwoliły na określenie ilości i składu olejków eterycznych i znalezienie zależności (także tych ukrytych) występujących między klasami wieku igieł.

* Corresponding author. E-mail: kuban@mendelu.cz; Fax: 420 5 4521 2044

Terpenes are main components of essential oils of coniferous trees. Concentration of several milligrams per gram of fresh weight is usually found in coniferous needles. Terpenes are stored mainly inside resin ducts [1]. They mediate many important interactions between trees and their environment. They have antibacterial and antifungal properties. Carbonylated monoterpenes are more active in a direct contact, while monoterpenes are more active in an ambient air. They can also repel or attract herbivores [2]. They are emitted from conifers to the atmosphere in large quantities and thus they play an important role in the atmospheric chemistry [3, 4].

The effects of seasonal environmental changes on growth and maturation of needles of Norway spruce [5] and *Pseudotsuga menziesii* [6] have been investigated. The effects of nutrient conditions [7], drought and water-logging [8] and air pollutants [9] on terpene content in coniferous needles have been investigated. An injury of a needle [1] or a trunk [7] can cause changes in the amount and the composition pattern of essential oils inside resin ducts as terpenes and stilbenes participate in wound healing. Also, long-term drought stress can induce the increased production of terpenes. The content of needle terpenes was generally diminished by ozone and acid mist treatment [9]. The changes in monoterpene concentration ratios in the needles of pollution-suffering *Picea abies* correlated with montane yellowing [1].

Composition pattern of essential oil is under genetic control, but high variability appears. The content of monoterpenes can also be related to the UV exposure, as well as to the location of a needle on the tree or on the whorl [10]. Schonwitz *et al.* [11] have compared the content of monoterpenes in 47 adult spruces growing under natural conditions in three locations. The total amount of terpenes varied from 50 to 70%, the variability due to different locations was insignificant. Variability in the composition pattern of oils was negligible. The variability among wild trees was higher than among the cloned ones [10]. Despite high variability, some differences among year classes were observed. Merk *et al.* [10] have observed some differences in the production of terpenes within whorls and needles depending on the age of a tree; there was no such difference in case of various locations on the branch. However, some differences have been found inside branches [11]. These results can not be generalised due to the short period of collection of experimental data (only 3 years old needles were inves-tigated).

The aim of this work was to determine the content and composition pattern of essential oils and their dependence on the needles' age, and also to find some latent interrelations using multivariate statistics methods. The main assumption in the selection of biological material was that the concentration of monoterpenes strongly depends on stress factors; consequently, the needles of different ages from the basal part of a tree growing under unusual environmental conditions were selected for the study. The stressors typical for northern and southern regions (low total annual rain precipitate and variable climatic factors) of natural appearance of *P. abies* strongly affect its growth in the pre-selected locality.

EXPERIMENTAL

Plant material

The pre-selected tree of *Picea abies (L.) Karst* from natural seedling in the onthogenic phase of early maturity (40 years old) was 10 m high. The tree of high vitality was grown in the open forest. The control tree grew in the southern part of Drahanská vrchovina, near Soběšice (in the North of Brno city, 420 m over sea level (o.s.l.), biotic granodiorite rocks, eutric Cambisol soil, mild warm and dry climatic conditions, total annual rain precipitation 490 mm, mean annual air temperature 8.7°C – climatological data averaged over the period 1961–1990). The samples were collected in February from the whorl situated at the height of approx. 3 m, *i.e.* from the base of the crown. Needles of different age classes were cut from the 1st order stem in the inner part of the crown. The collected samples contained the needles of different ages, *i.e.* from the oldest (1991 class) to the youngest (1999 class). The needles were healthy and without any observable color changes.

Sampling procedure

Needles from the main part of the whorl were separated according to their age, stored in boxes at 0°C and immediately transported to the laboratory and there stored also at 0°C. Individual groups of needles were treated with organic solvent as described in the literature [12]. Four analyses were done for each group of needles; for the group dating back 1991 only 2 analyses were performed (not enough needles were available). The total length of needles, dry matter weight (DMW) and the length of branches, from which the needles were collected, were measured. Non-identified monoterpenes were numbered. The numbers correspond to their retention times in chromatograms. Their quantity might differ from exact concentrations [mg g^{-1}].

Total daily, weekly, monthly and annual rain precipitates and mean temperatures in 1990–1999 were calculated on the basis of data obtained from the Czech Institute of Hydrometeorology in Brno. Concentrations and composition patterns of monoterpenes were determined in relation to the year of differentiation of needles (age of needles) and in relation to the characteristics of shoots: length and DMW. Their changes were attributed to variable climatic conditions.

Chemicals

Standard solutions of monoterpenes (α-pinene, β-pinene, 3-carene, camphene, α-phellandrene, limonene, *etc.*, all of p.a. purity) were purchased from Fluka. n-hexane (of reagent grade) was purchased from Merck (Darmstadt, Germany).

Chromatographic analysis

An HP–6890 gas chromatograph equipped with an HP–5673 mass spectrometric detector and an HP–INNOWax column (30 m × 0.25 mm × 0.5 μm polyethylene glycol film, Hewlett–Packard) was used for determination of monoterpenes. Flow rate of helium was 1.7 mL min^{-1} and injector temperature was 250°C. Split ratio of 5:1 was applied. The following temperature programme for quantitative analysis was applied: starting temperature 60°C, 5°C min^{-1} ramp up to 100°C, 30°C min^{-1} ramp up to 240°C, and 15 min at 240°C. An HP 5673 mass spectrometric detector was operated in the SIM mode and it was regularly calibrated with an HP calibrating solution. Spectra were recorded at 91, 93, 120 m/z, and also at 68 m/z starting from the 5th min to achieve better sensitivity for limonene.

Statistical data analysis

ADSTAT (TriloByte Statistical Software, Pardubice), STATGRAPHICS, SCAN, MINITAB programmes were used for statistical data treatment. Exploratory data analysis, ANOVA, principal component analysis, factors analysis, cluster analysis [14], and regression analysis were performed [15].

RESULTS AND DISCUSSION

Basic comments

The investigated needles can be divided into three distinct groups according the age of needles and the corresponding concentration of monoterpenes. Concentration of tricyclene in the needles plotted *vs* the year of growth formed an awed arch. The nearly increasing trend was observed for B-germarcene and terpene T2030. Lower content of B-germarcene was found in older classes compared to the younger ones. Similarly lower content of terpene T2030 was found in older classes (1991 and 1992) than in younger ones, in which it was almost constant. Age class from 1997 for B-germarcene and age classes from 1993 and 1997 for tricyclene and terpene T2030 significantly deviated from the trend. Generally, the classes from 1993 and 1997 deviated from the trend that was additionally confirmed by principal component analysis and factor analysis.

One performed growth analysis, in which the number of shoots and the length of branch were regressed *vs* the year of twig growth. Branch length steadily increased up to 1992, when very dry and hot summer occurred. Thus, only a small number of buds were formed as a basis for the next year (really less than can be expected according the trend). In 1993 the weather was also extremely dry, thus extremely low number of buds was formed again on twigs. In 1994 the weather was optimal, thus the number of buds was much higher than in the previous year. In 1995, which provided also very convenient conditions, the number of based twigs was decreased due to the aging of branches.

Exploratory data analysis

Pre-selected data sets mostly follow a skewed asymptotic distribution. Normal distribution applied only to datasets of α-phellandrene, limonene, terpinolene, and terpenes of retention times of 8.19 and 16.68 min. Rectangular distribution applied to datasets of B-germarcene and terpene of retention time 20.50. The lognormal distribution applied to the dataset of terpene of retention time 20.93. Distribution of datasets was determined using an ADSTAT programme on the basis of distribution with the highest correlation coefficient of the rankit Q–Q graph. Exploratory analysis has confirmed independency of all datasets.

Transformation of datasets

Datasets were appropriately transformed for the statistical treatment using Box–Cox and logarithmic (1 = 0) transformation. The found exponents 1 are given in Table 1. The corresponding data for DMW and shoot lengths were not transformed since normal distribution applied to both. Also the datasets of needle lengths were not transformed since only three corresponding discrete values were obtained.

Table 1. The calculated exponents 1 of data transformation

No.	Compound	λ	No.	Compound	λ	No.	Compound	λ
1	Tricyclene	0	7	α-phellandrene	0.93	13	T1712	0.13
2	α-Pinene	0	8	Limonene	0	14	Caryophylene	–0.13
3	Camphene	0	9	β-Phellandrene	0	15	T2050	0
4	β-Pinene	0	10	Terpinolene	0.67	16	T2068	0.13
5	Sabinene	0	11	T1668	0.53	17	T2093	0.27
6	Myrcene	0	12	Bornyl acetate	0	18	B-Germarcene	0.53

Analysis of correlation matrix

Correlation matrices for the transformed datasets of terpenes, twig lengths, needle lengths and DMW are presented in Tables 2a–2c. Datasets were divided into three groups on the basis of correlation results:

- tricyclene, α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, β-phellandrene, T1668, bornyl acetate, T1712, caryophylene, T2050, T2068 (correlation coefficients 0.75–1.00 and 0.74–1.00 for original and transformed data, respectively);
- terpinolene, α-phellandrene, B-germarcene (from 0.86 to 0.95 for both sets). The contents of terpenes correlated negatively with the data for shoots lengths (–0.65) and positively with the data for DMW (0.65 and 0.66 for original and transformed data, respectively). The data for needle lengths correlated negatively very well with the group (–0.60–(–0.75));
- T2093, original data of terpene contents did not correlate with any group.

The transformed data of terpenes correlated with both groups (up to 0.72 for the first and 0.85–0.95 for the second group). The behavior of terpene data after transformation was close to that of the second group. Linear regression could be applied due to the very high correlation between variables. Results of linear regression agreed very well with the results of graphical evaluation applying PCA–NIPALS (see the loading plot, Fig. 1.). Consequently, the relation between the monoterpene data could be clearly seen (see also a discussion bellow).

Table 2a. Correlation matrix – the transformed data

	Tricyklene	α-Pinene	Camphene	β-Pinene	Sabinene	Myrcene	α-Phellandrene
α-pinene	**0.998**						
Camphene	**1.000**	**0.998**					
β-pinene	**0.958**	**0.967**	**0.959**				
Sabinene	**0.951**	**0.952**	**0.949**	**0.953**			
Myrcene	**0.951**	**0.953**	**0.954**	**0.947**	**0.965**		
α-Phellandrene	0.358	0.368	0.374	0.469	0.400	0.521	
Limonene	**0.928**	**0.927**	**0.932**	**0.919**	**0.924**	**0.944**	0.625
β-Pphellandrene	**0.890**	**0.899**	**0.895**	**0.945**	**0.933**	**0.964**	0.637
Terpinolene	0.139	0.153	0.155	0.289	0.211	0.344	**0.961**
T1668	**0.834**	**0.843**	**0.840**	**0.864**	**0.815**	**0.882**	**0.710**
Bornyl acetate	**0.909**	**0.910**	**0.915**	**0.916**	**0.913**	**0.966**	**0.665**
T1712	**0.953**	**0.954**	**0.957**	**0.948**	**0.938**	**0.977**	0.575
Caryophylene	**0.986**	**0.984**	**0.989**	**0.946**	**0.921**	**0.948**	0.440
T2050	**0.737**	**0.744**	**0.746**	**0.806**	**0.815**	**0.880**	**0.794**
T2068	**0.985**	**0.983**	**0.985**	**0.942**	**0.911**	**0.917**	0.323
T2093	0.368	0.378	0.381	0.476	0.392	0.523	**0.975**
B-Germarcene	0.073	0.074	0.089	0.147	0.095	0.235	**0.892**
Twig length	–0. 043	–0. 071	–0. 055	–0. 256	–0.179	–0. 228	***–0.647***
Needles length	–0. 498	–0. 515	–0. 493	–0. 549	–0.536	–0. 451	–0.008
DMW	–0. 294	–0. 286	–0. 277	–0. 182	–0.282	–0. 134	0.558

Bold: correlation higher than 0.7.
Italic: correlation lower than –0.7.

Table 2b. Correlation matrix – the transformed data

	Limonene	β-Phellandrene	Terpinolene	T1668	Bornyl acetate	T1712	Caryophylene
β-Phellandrene	**0.926**						
Terpinolene	0.427	0.486					
T1668	**0.916**	**0.902**	0.558				
Bornyl acetate	**0.972**	**0.960**	0.491	**0.946**			
T1712	**0.973**	**0.953**	0.387	**0.931**	**0.986**		
Caryophylene	**0.940**	**0.904**	0.223	**0.865**	**0.931**	**0.958**	
T2050	**0.873**	**0.935**	**0.687**	**0.903**	**0.935**	**0.883**	**0.776**
T2068	**0.896**	**0.841**	0.105	**0.810**	**0.878**	**0.924**	**0.972**
T2093	0.608	**0.628**	**0.937**	**0.723**	0.655	0.580	0.440
B-Germarcene	0.384	0.355	**0.904**	0.520	0.434	0.321	0.181
Twig length	–0. 206	–0. 423	***–0. 717***	–0. 314	–0. 302	–0. 241	–0. 073
Needle length	–0. 392	–0. 434	0.087	–0. 339	–0. 389	–0. 451	–0. 411
DMW	–0. 121	0.045	**0.662**	0.034	–0. 016	–0. 118	–0. 169

Bold: correlation higher than 0.7.
Italic: correlation lower than –0.7.

Table 2c. Correlation matrix – the transformed data

	T2050	T2068	T2093	B-Germarcene	Twig length	Needle length
T2068	**0.691**					
T2093	**0.764**	0.330				
B-Germarcene	0.600	0.044	**0.845**			
Twig length	–0. 500	0.011	***–0. 648***	–0. 479		
Needle length	–0. 273	–0. 511	–0. 042	0.315	0.357	
DMW	0.211	–0. 333	0.537	**0.675**	–0. 532	0.537

Bold: correlation higher than 0.7.
Italic: correlation lower than –0.7.

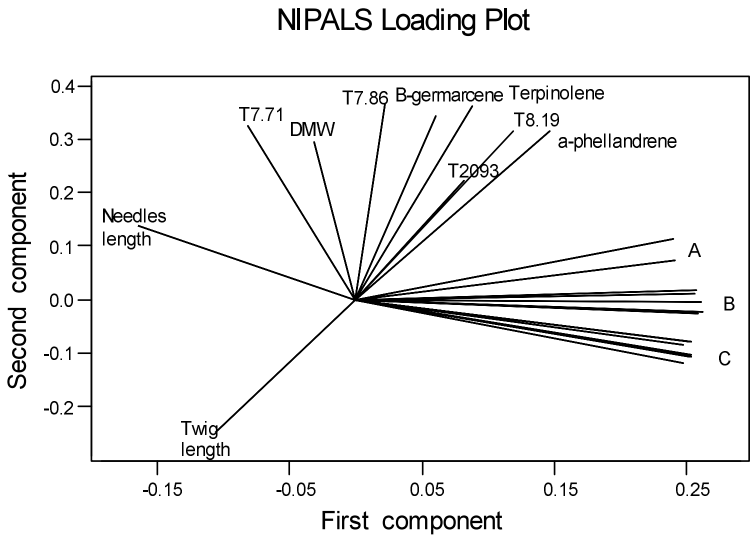


Figure 1. NIPALS – loading plot: group A – terpenes eluted from the column after 16.68 and 20.50 min; Group B – bornyl acetate, limonene, β-phellandrene, myrcene; Group C – caryophylene, tricyclene, α-pinene, camphene, β-pinene, sabinene, and terpene of retention time of 20.68 min. Very close correlation exists between B-germarcene, terpinolene, T2093 and α-phellandrene. DMW, needle lengths and twig lengths do not correlate with composition pattern of terpenes

Analysis of variance

One-way analysis of variance of the data after Box–Cox transformation for tricyclene (1. group), B-germarcene (2. group) and monoterpene T2093 (3. group) was performed. Age of needles (period: 1991–1999, n = 4, for 1991 n = 2) was as an investigated factor and it was used to confirm statistically a significant difference between age classes for all three terpenes. Quantil F (1–a, k–1, n–k) was 2.337 while the parameters using the F-test criterion were always higher and equalled 41.96, 40.91 and 19.38, respectively.

Principal component analysis – NIPALS

The results of data treatment using NIPALS for all terpenes are listed in Figures 1 and 2 (Score Plot, Loading Plot). They include twig lengths, needle lengths, and DMW. The trend observed for the first component was unclear. In the centre of the graph the data corresponding to 1993 can be distinguished – they indicate that 1993 was warm and dry (right low). In 1997 the spring was dry and there was a flood in the summer (left to the centre). The data corresponding to 1991 and 1992 are positioned in the lower left part of the figure. The data corresponding to the needles grown in the years with favorable weather are situated in the central part of the figure.

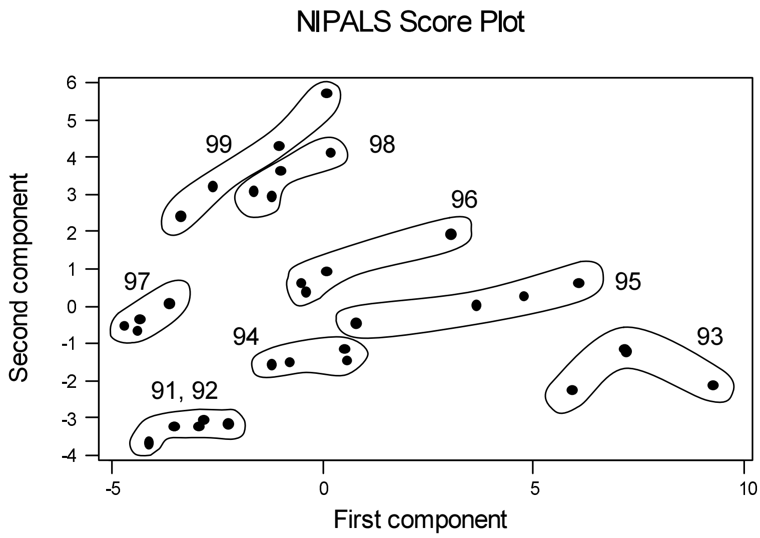


Figure 2. NIPALS – score plot: the elements from individual years are grouped; component 1 corresponds with the sum of terpenes in the needles; component 2 corresponds with the age of needles

The latent trend of the second component (age of needles) is also observable in Figure 2. The oldest classes were situated in the lower part (individual clusters from 1991 and 1992). The cluster of the youngest classes (1998 and 1999) was situated in the upper par, and other classes in the center. The clusters of individual year classes are oblong and are diagonally oriented. All the clusters exhibit the tendency to form closed formations, except those corresponding to 1997 and 1993.

Data in Figure 1 show that very close correlation exists between the terpenes. Individual loads are described in the figure. Apparently, the number of variables can be reduced in further analyses.

Correlations between terpenes were very closely related to the synthesis pathways [16] since monoterpene cyclases (synthases) have a similar reaction mechanism to prenyl transferases. The same enzymes controlled the pathways and also similar external effects initiated their synthesis, as it can be concluded from the similarities between monoterpenes (mainly in group C). The finding of Wagschal *et al.* (1994) that cyclase is responsible for the synthesis of terpenes in sage has been confirmed. Pinene cyclase I converted GPP into bicyclic (+)-α-pinene and (+)-camphene, cyclase II forms (–)-α-pinene and (–)-β-camphene, cyclase III transformed GPP into (+)-α-pinene, (+)-β-pinene, and monocyclic and acyclic olefins. Stereochemistry of these conversions has been already investigated [18]. These findings correspond well to the fact that α-pinene, β-pinene and camphene are present in the same group (Fig. 1).

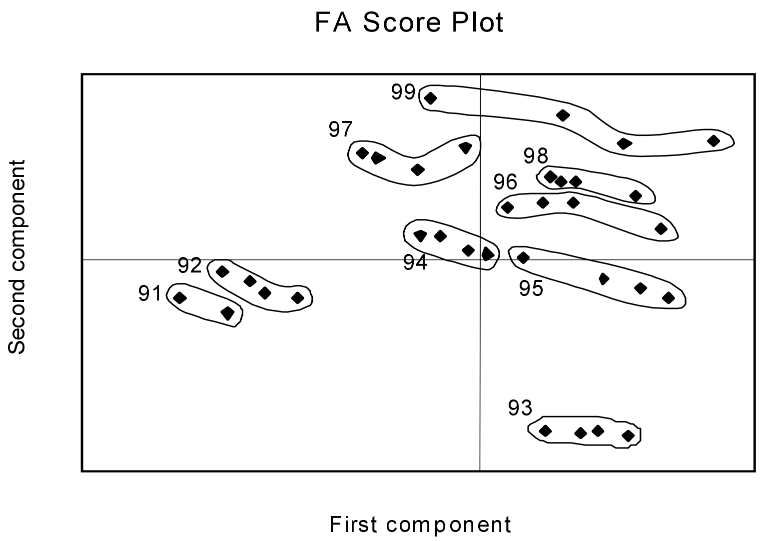
Myrcene correlated with α-pinene, β-pinene and limonene. This could be due to the fact that all were synthesised in the linalyl diphosphate pathway [17]. Myrcene was formed directly from linalyl diphosphate while three others required the formation of terpinyl cation from the linalyl diphosphate as an intermediate. Limonene, terpinolene, bornyl diphosphate, camphene, α-pinene, β-pinene, β-phellandrene, sabinene, and 3-carene were synthesised from α-terpinyl cation. This was in agreement with our results since all monoterpenes exhibited high correlation coefficients (up to 0.99). Although the literature data [17] concern sage and mint, we have concluded that similar enzymes and similar syntheses occur in the needles of *Picea abies*.

Factor analysis (FA)

Factor analysis was performed for three factors. Selected variables, that minimally correlated with each other, *i.e.* tricyclene, limonene, T16.68, terpinolene and B-germarcene (Fig. 3a–3c), have been chosen. The first component in factor analysis (Fig. 3a, only two components; 1 and 2, are depicted) corresponded to the total amount of terpenes in the needles (low concentrations on the left, and high on the right). The relationship between the content of terpenes in the needles and weather was discussed above. This first factor was not as significant for reduced number of variables as for the full matrix (including all terpenes). The second component corresponded to the age class and the third component (Fig. 3b and 3c) indicated the conditions during growth of the needles. The last component was used to divide the needles into the classes corresponding to the year: 1993, 1997 and 1999. For these years dry weather in April and/or May and/or June was characteristic. Two of these three months were dry and hot with rain precipitate levels below 40 mm per month and p/T ratio lower than 3, followed by a short „monsoon" Medard in July and dry rest of the summer.

The years 1991 and 1992 (and 1997 and 1999) were characterised by very similar weather conditions (the parameters are very close to each other in the figure) and formed one group. In contrast, the weather was different between 1997/1999 and 1991/1992. Very dry spring and very dry summer with scarce rains characterised the period 1997/1999. The period 1991/1992 was relatively dry. More precipitates appeared (compared to 1997/1999) during the needle-growing period in May/June.

Extremely low level of rain precipitates occurred in 1993, except from showers in the summer. Thus, the trees experienced a water stress over the whole year. The years 1994–1996 and 1998 (weather conditions were not optimum) did not follow the general trend but the growth of trees was satisfactory. Canonic correlations were not very helpful since statistically insignificant coefficients were obtained.



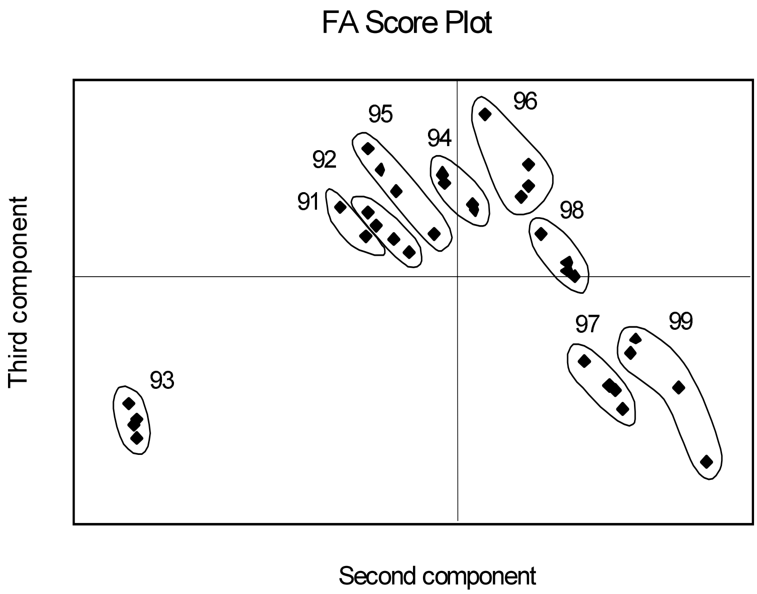


Figure 3. Factor analysis – score plot: **a.** components 1 and 2; component 1 corresponds with the sum of terpenes in the needles, component 2 corresponds with the age of needles; **b.** components 1 and 3; component 1 corresponds with the sum of terpenes in the needles, component 3 separates the needles according to the high (upper part of the figure) and low (lower part) stress caused by dry and hot conditions; **c.** components 2 and 3; component 2 corresponds with the age of needles, component 3 separates the needles according to the high (upper part of the figure) and low (lower part) stress caused by dry and hot conditions. (Continuation on the next page)

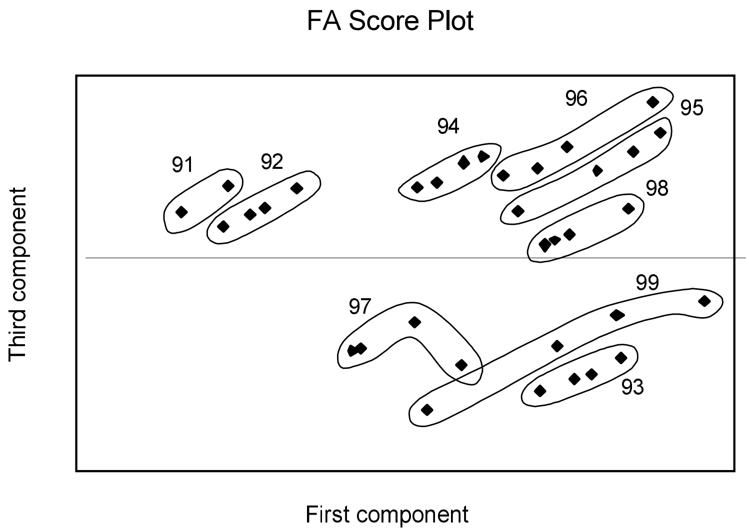


Figure 3. (Continuation)

Hierarchical clustering

Figure 4 presents the results of cluster analyses performed applying a Complete Linkage method. The closest clusters exhibited the shortest distance between the two most distant objects. A single digit corresponding to the last digit of the growth year number corresponded to individual groups of needles. The smallest clusters nearly corresponded to the age of needles. The groups corresponding to 1999, 1998, and 1996 were very similar (similarity 66%) to the group from 1995 using the next level. The same result was obtained for 1991 and 1992. Similarities between other groups were very low. For 1997 and 1994 individual groups of monoterpenes were formed. For 1993 another group was formed, which corresponded to the group from 1997 and 1994 at the 50% level of similarity. This finding partly corresponded with the Loading Plot (Fig. 1).

Figure 5 represents hierarchical clustering of variables. Terpenes could be separated into the following six groups: i) objects No. 2, 3, 8, 12: α-pinene, camphene, limonene, bornyl acetate, ii) 7, 10, 18: α-phellandrene, terpinolene, B-germarcene, iii) 1, 4, 6: tricyclene, β-pinene, myrcene, iv) 5, 9, 15: sabinene, β-phellandrene, T2050, v) 11, 16: T1668 and T2068, vi) 13, 17, 14: T1712, T2093, caryophylene.

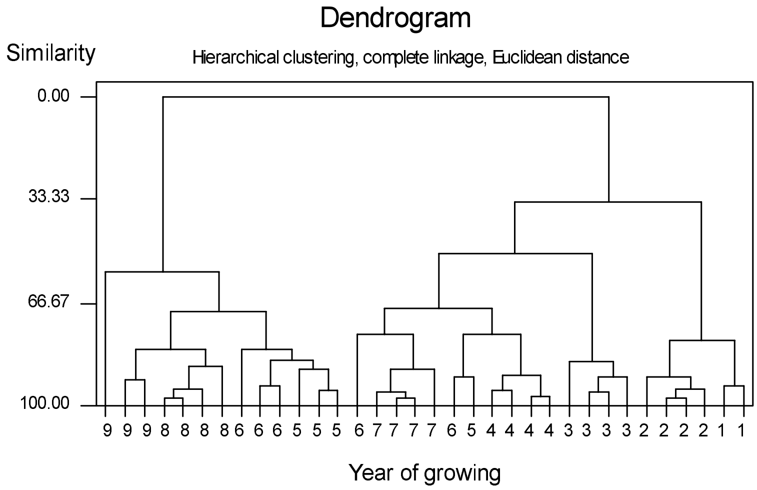


Figure 4. Dendrogram – year of growing similarity, complete linkage, Euclidean distance; year of growth: 1 – 1991, 2 – 1992, 3 – 1993, 4 – 1994, 5 – 1995, 6 – 1996, 7 – 1997, 8 – 1998, 9 – 1999

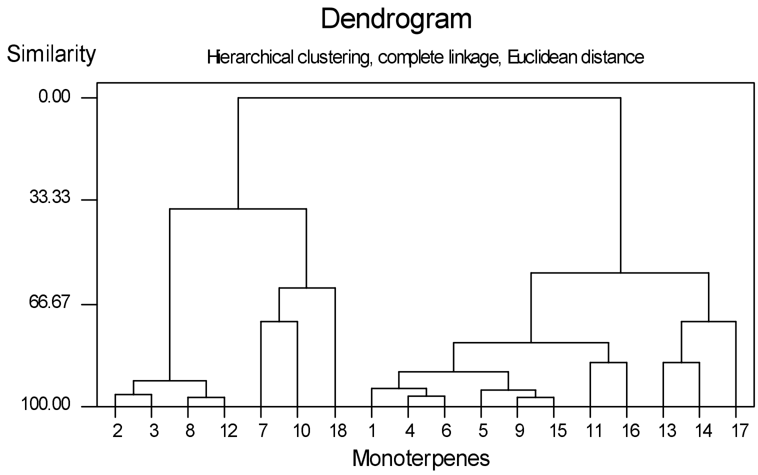


Figure 5. Dendrogram – year of growing similarity, complete linkage, Euclidean distance; legend: 1 – tricyclene; 2 – α-pinene; 3 – camphene; 4 – β-pinene; 5 – sabinene; 6 – myrcene; 7 – α-phellandrene; 8 – limonene; 9 – β-phellandrene; 10 – terpinolene; 11 – T1668; 12 – – bornyl acetate; 13 – T1712; 14 – caryophylene; 15 – T2050; 16 – T2068; 17 – T2093; 18 – B-germarcene

First two groups of monoterpenes were distinct, while other monoterpenes formed one large group. The same results were obtained using other statistical methods. Two groups were identical at the highest level of similarity, while the contents of ii) could be either transferred from one group to the other, or it formed a separate group at the 50% level, or at lower levels.

CONCLUSIONS

In the middle European region, spruce is planted as a very effective and economically interesting species, although its growing conditions are far from optimum. It is obvious that the concentration of secondary metabolites (mainly essential oils) follows negative stress factors mainly at the height lower that 500 m over the sea level. Spruce is affected by stressors typical for northern and southern areas of its natural appearance (irregular early and later freezes, exceptionally mild and/or very cold winters, wet and cold or hot and dry summers, *etc.*).

The influence of climatic conditions on the contents of individual monoterpenes in spruce needles in dependence on their age, size, and conditions (mainly dryness during elongation periods of whorls) has been investigated. Also similarities and differences between synthesis pathways of individual monoterpenes are important. For the above reasons, the site representing the most irregular growing conditions for spruce (relatively low annual rain precipitates, changeable climatic factors) was chosen. A tree without any visual damage (*i.e.* yellowing of needles, *etc.*) was selected for this study. The needles from the oldest, basal part of the tree were collected for the analysis. For these needles less sharply expressed changes in bio-climatic conditions were expected compared to highly illuminated parts of the tree crown. Multivariate statistical methods, namely the principal component analysis, correlation analysis, cluster analysis, and ANOVA were applied to specify the amount and composition pattern of essential oils, their dependence on the needles' age, and some latent relationships.

Composition patterns of monoterpenes were found to be dependent on the year of differentiation of needles (age of needles) and on the characteristics of shoots (length and total dry matter weight, DMW).

Acknowledgment

Financial support from the Grant Agency of the Czech Republic (grant No. 203/98/0943, 526/03/1182) is gratefully acknowledged.

REFERENCES

1. Jűttner F., *Physiol. Plantarum*, **72**, 48 (1988).
2. Naigre R., Kalck O., Roques Ch., Roux I. and Michel G., *Planta Medica*, **62**, 275 (1996).
3. Večeřa Z., *Chem. Listy*, **95**, 157 (2001).

4. Schurmann W., Ziegler H., Kotzias D., Schönwitz R. and Steinbtecher R., *Naturwis.*, **80**, 276 (1993).
5. Schönwitz R., Lohwasser K., Kloos M. and Ziegler H., *Trees*, **4**, 34 (1990).
6. Zou J. and Cates R.G., *J. Chem. Ecol.*, **21**, 387 (1995).
7. Viiri H., Annila E., Kitunen V. and Nimelä P., *Trees*, **15**, 112 (2001).
8. Kainulainen P., Oksanen J., Palomäki V., Holopainen J. K. and Holopainen T., *Can. J. Bot.*, **70**, 1613 (1992).
9. Heller W., Roseman D., Osswald W. F., Benz B., Schonwitz R., Lohwasser K., Kloos M. and Sandermann H., *Environ. Pollut.*, **64**, 353 (1990).
10. Merk L., Kloos M., Schönwitz R. and Ziegler H., *Trees*, **2**, 45 (1988).
11. Schönwitz R., Kloos M., Merk I. and Ziegler H., *Trees*, **4**, 27 (1990).
12. Holubová V., Chvílíčková I. and Kubáň V., *Collect. Czech. Chem. Commun.*, **65**, 1073 (2000).
13. Holubová V., Hrdlička P. and Kubáň V., *Phytochem. Anal.*, **12**, 243 (2001).
14. Meloun M., Militký J. and Forina M., *Chemometrics for Analytical Chemistry*, Vol. 1. PC-Aided Statistical Data Analysis, Ellis Horwood, Chichester, 1992, p. 212.
15. Meloun M., Militký J. and Forina M., *Chemometrics for Analytical Chemistry*, Vol. 2. PC-Aided Regression and Related Methods, Ellis Horwood, Chichester, 1994.
16. Dey P.M. and Harborne J.N., *Plant Biochemistry*, Academic Press, London, 1997, p. 420.
17. Leeper F.J. and Veredas J.C., *Biosynthesis, Aromatic Polyketides, Isoprenoids, Alkaloids*, Springer Vrlg., 2000, pp. 60.
18. Pyun H.-J., Wagschal K.C., Jung D.-I., Coates R.M. and Croteau R., *Arch. Biochem. Biophys*., **308**, 488 (1994).
19. Karpov V.G. (Ed.), *Struktura i produktivnost jelovych lesov juzhnoj tajgy*, NAUKA, 1973, Leningrad.

Received April 2006
Accepted June 2006