© Verlag Alexander Just: Dornbeuern - Salzburg - Brüssel; download unter www.biologiezentrum.at

COMPOSITION OF VOLATILES FROM NEEDLES OF *PINUS NIGRA* ARNOLD, *P. SYLVESTRIS* L. AND THEIR HYBRIDS

Die Zusammensetzung der flüchtigen Nadelinhaltsstoffe von *Pinus nigra* ARNOLD, *P. sylvestris* L. und ihrer Hybriden

by

Marilena IDŽOJTIĆ & Hartwig W. PFEIFHOFER

Key words: *Pinus nigra* ARNOLD, *P sylvestris* L., interspecific hybrids, volatiles, GC, GC/MS, terpenes, cluster analysis.

Schlagwörter: *Pinus nigra* ARNOLD, *P. sylvestris* L., interspezifische Hybriden, flüchtige Substanzen, GC, GC/MS, Terpene, Clusteranalyse.

Summary: The composition of needle volatiles of *Pinus nigra* ARNOLD (=ni), *P sylvestris* L. (=sy) and their F_1 and F_2 hybrids (=F_1 nisy, F_2 nisy) was investigated with gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The number of detected components varied with species and hybrids (ni 141, sy 107, F_1 nisy 129, F_2 nisy 83). A total of 55 components was identified, of which 51 were unambigously identified and 4 were partially characterized. The identified components accounted for 94.4% w/w sample of *P. nigra,* 90.1% w/w sample of *P. sylvestris*, 91.4% w/w sample of F_1 hybrids, and 93.9% w/w sample of F_2 hybrids. All identified components were terpenes except *trans*-2-hexenal which is a n-alkene. The amount of monoterpenes was the highest (ni 65%, sy 54%, F_1 nisy 64%, F_2 nisy 71%), followed by sesquiterpenes (ni 27%, sy 36%, F_1 nisy 27%, F_2 nisy 23%) and diterpenes (ni 2.34%, sy 0.2%, F_1 nisy 0.11%, F_2 nisy 0.16%). Cluster analysis shows that the hybrids F_1 and F_2 are the most similar, and that both hybrid groups are more similar to European black pine *(P. nigra)* than to Scots pine *(P. sylvestris).*

Zusammenfassung: Die Zusammensetzung der flüchtigen Nadelinhaltsstoffe von *Pinus nigra* ARNOLD (=ni), *P. sylvestris* L. (=sy) und ihrer F_1- und F_2-Hybriden (=F_1 nisy, F_2 nisy) wurde mittels Gaschromatographie (GC) und Gaschromatographie/Massenspektrometrie untersucht. Die Anzahl nachgewiesener Verbindungen schwankte je nach Art und Hybride (ni 141, sy 107, F_1 nisy 129, F_2 nisy 83). 51 von insgesamt 55 Verbindungen konnten eindeutig identifiziert werden, 4 wurden teilweise charakterisiert. Die identifizierten Verbindungen machten 94.4 Gewichtsprozent (%) des Nadelöls von *Pinus nigra,* 90.1% von *P. sylvestris,* 91.4% der F_1-Hybriden und 93.9% des Nadelöls der F_2-Hybriden aus. Mit Ausnahme des Aldehydes *trans*-2-Hexenal waren alle identifizierten Verbindungen Terpene. Monoterpene hatten den größten Anteil (ni 65%, sy 54%, F_1 nisy 64%, F_2 nisy 71%), gefolgt von Sesquiterpenen (ni 27%, sy 36%, F_1 nisy 27%, F_2 nisy 23%) und Diterpenen (ni 2.34%, sy 0.2%, F_1 nisy 0.11%, F_2 nisy 0.16%). Eine Clusteranalyse ergab, dass die F_1- und F_2-Hybriden sich am ähnlichsten sind und dass beide Hybridgruppen der Schwarzkiefer *(P. nigra)* ähnlicher sind als der Rotkiefer *(P. sylvestris).*

Introduction

The F_1 and F_2 hybrids *Pinus nigra* ARNOLD × *P sylvestris* L. have been produced at the Department of Forest Genetics and Dendrology of the Faculty of Forestry, University of Zagreb, Croatia.

The F_1 hybrids (=F_1 nisy) were produced by the controlled hybridization from 1968 to 1989. The successful crossing was possible only with the European black pine as female parent and the Scots pine as male parent *(P. nigra × P. sylvestris).* These hybrids were analyzed from various aspects in several papers, under the name of *P × nigrosylvis* VID. (VIDAKOVIĆ, 1977a,b, 1983, 1986; VIDAKOVIĆ & BORZAN, 1991; BORZAN, 1984, 1987, 1988; BORZAN et al., 1995; BORZAN & IDŽOJTIĆ, 1996; IDŽOJTIĆ, 1996, 1997).

By the controlled hybridization of F_1 hybrids *((P nigra* × *P* sylvestris) × *(P nigra* × *P sylvestris)),* in 1984 and 1985 the hybrids of F_2 generation were produced (=F_2 nisy). BORZAN & IDŽOJTIĆ (1996) and IDŽOJTIĆ (1996, 1997) reported about the morphology of needles in the F_2 hybrids, while their growing in the experimental plots was reported on the XX IUFRO Congress in Finland (BORZAN et al., 1995).

The hybrids were planted in the plots in Croatia (the Faculty of Forestry, Đurđevački peski and Arboretum Lisičine).

In this paper the composition of needle volatiles from hybrids in relation to the parental species has been researched.

The basic needle volatile components of pine species are terpenes. The terpene composition was used in chemotaxonomy and in the research of geographic variability for *P nigra* by GERBER et al. (1995), CHALCHAT &

GORUNOVIC (1995a,b), and for *P. sylvestris* by TOBOLSKI & HANOVER (1971), JUVONEN & HILTUNEN (1972), HILTUNEN *et al.* (1975), FORREST (1980), CHALCHAT *et al.* (1985), YAZDANI et al. (1985), MUONA et al. (1986), YAZDANI & NILSSON (1986), Orav et al. (1996).

GALLIS & PANETSOS (1997) researched the possibility to distinguish *Pinus brutia* TEN., *P. halepensis* MILL. and their F_1, F_2 and back crossed hybrids on the basis of the cortical oleoresin terpene composition. There were no qualitative differences, however quantitative differences in some important terpenes could be used to differentiate the species and their hybrids.

Material and methods

Plant Material and Sample Preparation

Needles were taken in October 1996, from fully developed one-year old shoots. Shoots of European black pine were taken from 41 trees and those of Scots pine from 29 trees, of F_1 hybrids from 32 trees and of F_2 hybrids from 35 trees. For each species, i.e. hybrid, 14-25 g of needles were distilled in a Karlsruher-type apparatus (STAHL, 1953) for six hours. Volatiles were collected into 1 ml n-pentane.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The composition of needle volatiles was investigated with gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

GC profiles were established on a DANI 8610 and DANI 8400 Capillary Gas Chromatograph (DANI, Monza, Italy) each equipped with a Programmed Temperature Vaporizer (PTV) injection system, a flame ionization detector (FID) and a LDC/Milton Roy CI-10 B integrator (LDC/Milton Roy, Riviera Beach, Florida). The samples were analyzed on fused silica capillary columns with bonded phases of different polarity.

The non-polar system comprised a CP-Sil 5 CB (dimethylpolysiloxane; 50 m x 0.22 mm i.d.; film thickness 0.13 µm) capillary column (Chrompack International BV, Middelburg, The Netherlands). Carrier gas (hydrogen) velocity was 43 cm/s. Column temperature programming was: 40 – 300°C at 4°C/min and 300°C isothermally for 10 min. PTV temperature was 50°C

during injection, followed by a very rapid heating to 280°C. The FID was operated at 310°C.

The polar system included a DB-Wax (polyethylene glycol; 60 m x 0.32 mm i.d.; film thickness 0.25 μm) capillary column (J & W Scientific, Folsom, California). Carrier gas (hydrogen) velocity was 53 cm/s. Column temperature programming was: 40°C held for 5 min, and then from 40°C heated at 2.5°C/min to 250°C. PTV temperature was 50°C during injection, followed by a very rapid heating to 250°C. The FID was operated at 260°C.

GC/MS was performed on a Hewlett Packard (Hewlett Packard, Paolo Alto, California) G 1800A GCD system (Electron impact voltage: 70 eV, interface temperature 320°C, mass range 30-425 amu). The samples were analyzed on a DB-1 (dimethylpolysiloxane; 50 m x 0.20 mm i.d.; film thickness 0.33 μm; J & W Scientific) capillary column. Other chromatographic conditions were: Carrier gas (helium) at 1 mL/min. Column temperature programming: 50°C initial temperature held for 3 min, then from 50 °C heated at 4°C/min to 320°C, 320°C was held for 5 min.

Compounds were identified using both chromatographic and mass spectroscopic criteria. The Wiley275 Data base was used for automatic identification of GC/MS peaks. Additionally, linear retention indices according to VAN DEN DOOL & KRATZ (1963) obtained on a polar and an apolar column were compared with published data (DAVIES, 1990). Mass spectra and retention indices were also compared with data obtained from authentic compounds. Quantification was achieved from GC-FID profiles on apolar column according to the area percent method without consideration of calibration factors (F), i.e. F=1.0 for all compounds.

Cluster Analysis

To determine the degree of similarities between analyzed species and hybrids, the area percent values were subject cluster analysis (tree clustering algorithm, Euclidean distance, complete linkage method).

Results and Discussion

The number of detected components varied with species and hybrids (*P. sylvestris* 107, *P nigra* 141, F_1 nisy 129 and F_2 nisy 83). A total of 55 components was identified, of which 51 were unambigously identified and 4 were partially characterized. Those 55 components are shown in Table 1.

© Verlag Alexander Just: Dorfbeuern - Salzburg - Brüssel; download unter www.biologiezentrum.at

They accounted for 90.1% w/w sample of *P. sylvestris*, 94.4% w/w sample of *P. nigra*, 91.4% w/w sample of F_1 hybrids and 93.9% w/w sample of F_2 hybrids. The residual to 100% were components which could not be identified, because they were present in the sample in such low amounts, that their mass spectra were too poor for interpretation.

Scots pine, *Pinus sylvestris* L.

A total of 107 components was detected, of which 47 were identified (comprising 89.4% w/w of the sample), and 3 were partially characterized (0.7% w/w of the sample).

All identified components are terpenes (89.98%), except *trans*-2-hexenal (0.12%), which is a n-alkene. The main substance class were monoterpenes (27), comprising 54.18%. Main constituents of the monoterpenes were α-pinene, 32.91% and Δ-3-carene, 8.86% (Figure 1 and 2). The second main substance class were sesquiterpenes (21), of which 18 were identified (34.88%), and 3 partially characterized (0.7%). Main constituents of the sesquiterpenes were *endo*-1-bourbonanol, 9.86%; α-muurolene, 4.3%; β-caryophyllene, 4.05%; δ-cadinene, 3.9%, germacrene D, 3.45% and γ-cadinene, 2.7% (Figure 1, 2, 3, 4). Two diterpenes were identified, 13-epimanoyl oxyde, 0.2% (Figure 4) and thunbergol, in traces.

So far, two chemotypes of *P. sylvestris* have been described, which differ significantly in their content of Δ-3-carene (CHALCHAT et al., 1985; ORAV et al., 1996). The Scots pine trees autochtonous in Croatia, whose needles were analyzed in this paper, seem to belong to the chemotype with a low Δ-3-carene content. The amount of Δ-3-carene in Scots pine from Estonia (23.7%) is significantly higher, since it belongs to the other chemotype (ORAV et al., 1996). There is also a difference in the amount of caryophyllene oxyde, which was not detected in our sample while in the Estonian sample it participated with 4.9%.

KUBECZKA & SCHULTZE (1987) analyzed the composition of essential oils in needles of six different pine species, among them Scots pine and European black pine. For the Scots pine they reported a somewhat higher amount of α-pinene (42%) and β-phellandrene + limonene (5.5%), than in this paper. The amount of Δ-3-carene was also high (20.6%), indicating that the sample originated from a Scots pine of the high Δ-3-carene chemotype. However, the provenance of the samples was not reported by the authors.

download unter www.biologiezentrum.at

European black pine, *Pinus nigra* ARNOLD

A total of 141 components was detected, of which 49 were identified (comprising 94.17% w/w of the sample), while 4 were partially characterized (0.23% w/w of the sample).

All identified components are terpenes (94.3%), except *trans*-2-hexenal (0.1%), which is a n-alkene. The main substance class were monoterpenes (28), comprising 64.88%. Main constituents of the monoterpenes were α-pinene, 42.66%, β-pinene, 11.64% and β-phellandrene + limonene, 3.66% (Figure 1 and 3). The second main substance class were sesquiterpenes (22), of which 18 were identified (26.85%), and 4 partially characterized (0.23%). Main constituents of the sesquiterpenes were germacrene D, 17.72% and β-caryophyllene, 5.62% (Figure 1 and 2). Two diterpenes were identified, 13-epimanoyl oxyde, 2.25% (Figure 4) and thunbergol, 0.09%.

CHALCHAT & GORUNOVIC (1995a) studied the composition of the essential oil of *P. nigra* ARNOLD ssp. *nigra* (ARN., HAYEK) from Bosnia and Herzegovina. A larger number of components (91) than in our study was identified, comprising 90% w/w of the sample. In addition to the qualitative difference existing between the identified components, the greatest quantitative difference is in the amount of germacrene D, which was reported to be present in traces. In the sample analyzed in this work, germacrene D is the second component by its amount (17.7%), after α-pinene. Needle volatiles analyzed by CHALCHAT & GORUNOVIC (1995a) had a higher amount of α-pinene (66.5%) and β-phellandrene + limonene (6%), and a lower amount of β-pinene (5.3%), than in our study.

The quantitative terpene pattern of European black pine described in this paper is similar to results presented by KUBECZKA & SCHULTZE (1987). The amount of germacrene D (18.6%) is equal to that in our sample (17.7%) and by its amount it is also the second component after α-pinene. Some qualitative differences exist however, because KUBECZKA & SCHULTZE (1987) mention only 25 components, whereas in our study more minor compounds could be identified.

F_1 hybrids *(P. nigra* × *P. sylvestris)*

A total of 129 components was detected, of which 50 were identified (comprising 91.11% w/w of the sample), and 4 were partially characterized (0.29% w/w of the sample).

All identified components are terpenes (91.27%), except *trans*-2-hexenal (0.13%), which is a n-alkene. The main substance class were monoter-

penes (28), comprising 64.37%. Main constituents of the monoterpenes were α-pinene, 36.24%; Δ-3-carene, 5.41%; camphene 5.34%; β-pinene, 5.31% and bornyl acetate 2.99% (Figure 1, 2, 3). The second main substance class were sesquiterpenes (23), of which 19 were identified (26.53%) and 4 partially characterized (0.29%). Main constituents of the sesquiterpenes were germacrene D, 11.6%; β-caryophyllene, 4.66%; *endo*-1-bourbonanol 3.01%; δ-cadinene 1.62% and α-muurolene 1.18% (Figure 1, 2, 3). Two diterpenes were identified, 13-epimanoyl oxyde, 0.11% (Figure 4) and thunbergol, in traces.

F_2 hybrids ((*P. nigra* × *P. sylvestris*) × (*P. nigra* × *P. sylvestris*))

A total of 83 components were detected, of which 49 were identified (comprising 93.64% w/w of the sample) and 3 were partially characterized (0.26% w/w of the sample).

All identified components are terpenes (93.79%), except *trans*-2-hexenal (0.11%), which is a n-alkene. The main substance class were monoterpenes (28), comprising 70.59%. Main constituents of the monoterpenes were α-pinene, 45.14%; camphene 5.5%; β-pinene, 4.9%; bornyl acetate 3.84%; myrcene 2.34%; Δ-3-carene, 2.32% and β-phellandrene + limonene, 2.18% (Figure 1, 2, 3, 4). The second main substance class were sesquiterpenes (21), of which 18 were identified (22.88%) and 4 partially characterized (0.26%). Main constituents of the sesquiterpenes were germacrene D, 10.91%; β-caryophyllene, 3.55%; *endo*-1-bourbonanol 3.07% and δ-cadinene 1.16% (Figure 1, 2, 3). Two diterpenes were identified, 13-epimanoyl oxyde, 0.04% (Figure 4) and thunbergol, 0.02%.

The volatile needle oils of the hybrids contain the same compounds as their parental species. However, there are quantitative differences between the essential oils of the individual groups. The same was also found out for the hybrids *P. brutia* × *P. halepensis* by GALLIS & PANETSOS (1997).

In Figures 1 – 4, the concentrations of main components with an amount exceeding 10% (Figure 1), 5% (Figure 2), 3% (Figure 3) and 1% (Figure 4) for at least one analyzed group (species or hybrid) are compared.

Components with a significantly higher amount in Scots pine than in European black pine needle volatiles are: *endo*-1-bourbonanol (sy 9.86%, ni 0.58%), Δ-3-carene (sy 8.86%, ni 0.2%), α-muurolene (sy 4.3%, ni 0.26%), γ-cadinene (sy 2.7%, ni 0.25%), T-muurolol (sy 2.41%, ni 0.27%) and α-cadinol (sy 2.07%, ni 0.23%). The other group are components with a considerably higher amount in European black pine than in Scots pine needle volatiles,

namely: germacrene D (sy 3.45%, ni 17.72%), β-pinene (sy 1.93%, ni 11.64%), β-phellandrene + limonene (sy 1%, ni 3.66%) and 13-epimanoyl oxyde (sy 0.2%, ni 2.25%).

The amount of most components in the F_1 and F_2 hybrids is intermediate as compared to the parental species.

In all four analyzed groups (species and hybrids), α-pinene had the highest concentration (sy 32.9%, ni 42.7%, F_1 nisy 36.24% and F_2 nisy 45.14%). The amount of this monoterpene in the F_1 hybrids was intermediate compared to the parental species, while in the F_2 hybrids it was higher than in both parental species.

The amount of β-pinene, germacrene D, Δ-3-carene, *endo*-1-bourbonanol, β-phellandrene + limonene, α-muurolene, γ-cadinene, α-cadinol and T-muurolol in the F_1 and F_2 hybrids is intermediate in relation to the parental species.

The amount of *trans*-β-ocymene and β-caryophyllene is intermediate in F_1 hybrids and lower in F_2 hybrids than in both parental species. F_1 hybrids contain more α-terpinolene than both parental species, while its content is intermediate in F_2 hybrids.

Needle oils of F_1 and F_2 hybrids have a higher concentration of the diterpene 13-epimanoyl oxyde than those of both parental species.

To determine the degree of similarity or difference between the analyzed species and hybrids according to the composition of needle volatiles, cluster analysis was applied (tree clustering algorithm, Euclidean distance, complete linkage method). The input data were percent values of detected components, which could be monitored parallelly for all four groups.

In Figure 5, the horizontal hierarchical tree (dendrogram) is given for four analyzed groups. According to the composition of needle volatiles, the most similar are F_1 and F_2 hybrids (linkage distance 9.8). Cluster made by hybrids is linked with the European black pine at a distance of 14.6, and then at a distance of 25.3 the Scots pine is joined. This means that by the composition of their needle volatiles the F_1 and F_2 hybrids are more similar to the European black pine, which is the female parent of F_1 hybrids, than to the Scots pine.

Conclusion

© Verlag Alexander Just: Dorfbeuern - Salzburg - Brüssel; download unter www.biologiezentrum.at

So far, cytological and morphometrical researches dealing with European black pine and Scots pine hybrids, produced at the Faculty of Forestry in Zagreb, has been made. Their growth (survival and increment) in the plots was monitored too. In this paper, the composition of needle volatiles of parental species and their hybrids was analyzed by GC and GC/MS.

The number of detected components was 107 for *P. sylvestris*, 141 for *P. nigra*, 129 for F_1 nisy and 83 for F_2 nisy. A total of 55 components was identified, of which 51 were unambigously identified and 4 were partially characterized. They accounted for 90.1% w/w sample of *P. sylvestris*, 94.4% w/w sample of *P. nigra*, 91.4% w/w sample of F_1 hybrids and 93.9% w/w sample of F_2 hybrids. All identified components were terpenes except *trans*-2-hexenal which is a n-alkene. The amount of monoterpenes was the highest (ni 65%, sy 54%, F_1 nisy 64%, F_2 nisy 71%), followed by sesquiterpenes (ni 27%, sy 36%, F_1 nisy 27%, F_2 nisy 23%) and diterpenes (ni 2.34%, sy 0.2%, F_1 nisy 0.11%, F_2 nisy 0.16%).

The needle volatiles of parental species and their hybrids show the same qualitative terpenoid pattern. However, there are quantitative differences between the essential oils of the individual groups. The amount of most components in the F_1 and F_2 hybrids is intermediate as compared to the parental species.

Cluster analysis shows that the hybrids F_1 and F_2 are the most similar, and that both hybrid groups are more similar to European black pine *(P. nigra)* than to Scots pine *(P sylvestris)*.

References

BORZAN, Ž. (1984): Hybridization between *Pinus nigra* and *Pinus sylvestris* and morphology of chromosomes. Proc. of the 8th international symposium on sexual reproduction in seed plants, ferns and mosses, Wageningen, 181p.

BORZAN, Ž. (1987): Oplemenjivanje četinjača. Glas. šum. pokuse, posebno izd. **3**: 243-253.

BORZAN, Ž. (1988): Kariotipovi nekih borova podsekcije *Sylvestres*. Glas. šum. pokuse **24**: 1-100.

BORZAN, Ž. & M. IDŽOJTIĆ (1996): Razlikovanje crnog bora *(Pinus nigra* ARN.), običnog bora *(P. sylvestris* L.) i njihovih F_1 i F_2 hibrida pomoću diskriminantne analize. U: Skrb za hrvatske šume od 1846. 1996.,

knjiga 1: Unapređenje proizvodnje biomase šumskih ekosustava (B. MAYER, urednik), 23-36 pp.

BORZAN, Ž., M. IDŽOJTIĆ & M. VIDAKOVIĆ (1995): Experimental plots of some hard pine hybrid families in Croatia. Ann. Forest. **20/1**: 1-36.

CHALCHAT, J.C. & M.S. GORUNOVIC (1995a): Chemotaxonomy of pines native to the Balkans. Part 2: Illyrian black pine *Pinus nigra* ARNOLD ssp. *nigra* (ARN., HAYEK), *Pinaceae*, according to location, plant part and age of specimens. Pharmazie **50 (4)**: 281-283.

CHALCHAT, J.C. & M.S. GORUNOVIC (1995b): Chemotaxonomy of pines native to the Balkans. Part 3: Variations in the composition of essential oils of the *Pinus nigra* ARNOLD ssp. *dalmatica* according to age of specimens. Pharmazie **50 (8)**: 575-576.

CHALCHAT, J.-C., GARRY R.-P., MICHET, A. & A. REMERY (1985): The essential oils of two chemotypes of *Pinus sylvestris*. Phytochemistry **24 (10)**: 2443-2444.

DAVIES, N.W. (1990): Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20 M phases. J. Chromatogr. **503**: 1-24.

FORREST, G.I. (1980): Genotypic variation among native Scots pine populations in Scotland based on monoterpene analysis. Forestry **53**: 101-128.

GALLIS, A.T. & K.P. PANETOSIS (1997): Use of cortical terpenes to discriminate *Pinus brutia* (Ten.), *Pinus halepensis* (Mill.) and their hybrids. Silvae Genet. **46 (2-3)**: 82-88.

GERBER, S., BARADAT, P., MARPEAU, A. & M. ARBEZ (1995): Geographic variation in terpene composition of *Pinus nigra* ARN. Forest Genetics **2 (1)**: 1-10.

HILTUNEN, R., JUVONEN, S. & P.M.A. TIGERSTED (1975): Geographical variation in some monoterpenes in Scots pine *(Pinus silvestris* L.) in Finland. Farm. Aik. **84**: 73-82.

IDŽOJTIĆ, M. (1996): Morfološka obilježja i uspijevanje nekih dvoigličavih međuvrsnih hibrida borova na pokusnim plohama Đurđevački peski i u Arboretumu Lisičine. Glas. šum. pokuse **33**: 301-338.

IDŽOJTIĆ, M. (1997): Morphological characteristics of the needles of European black pine *(P. nigra* ARNOLD), Scots pine *(P. sylvestris* L.), Japanese red pine *(P. densiflora* SIEB. et ZUCC.) and their interspecific hybrids. Ann. Forest. **22/2**: 29-59.

JUVONEN, S. & R. HILTUNEN (1972): Über das Vorkommen von 3-Caren-armen und -reichen Chemotypen bei *Pinus sylvestris* L. in Finland. Biogenetische Studien II. Farm. Aikak. **81**: 137-145.

Zobrazeno z repozitáře Ústavu pro lesní ekologii Přírodovědecké fakulty Masarykovy univerzity v Brně, http://www.ilec.muni.cz

KUBECZKA, K.-H. & W. SCHULTZE (1987): Biology and chemistry of conifer oils. Flavour Fragr. J. **2**: 137-148.

MUONA, O., HILTUNEN, R., SHAW, D.V. & E. MORÉN (1986): Analysis of monoterpene variation in natural stands and plustrees of *Pinus sylvestris* in Finland. Silva Fenn. **20**: 1-8.

ORAV, A., KAILAS, T. & M. LIIV (1996): Analysis of terpenoic composition of conifer needle oils by steam destillation/extraction, gas chromatography and gas chromatography-mass spectrometry. Chromatographia **43 (3/4)**: 215-220.

STAHL, E. (1953): Eine neue Apparatur zur gravimetrischen Erfassung kleinster Mengen ätherischer Öle. Mikrochemie **40**: 367-372.

TOBOLSKI, J.J. & J.W. HANOVER (1971): Genetic variation in the monoterpenes of Scotch pine *(Pinus sylvestris* L.). For. Sci. **17**: 293-299.

VAN DEN DOOL, H. & P.D. KRATZ (1963): A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. **11**: 463-471.

VIDAKOVIĆ, M. (1977a): Some morphological characteristics of *Pinus* × *nigrosylvis (P. nigra* × *P. sylvestris),* Ann. Forest. **8/2**: 15-27.

VIDAKOVIĆ, M. (1977b): The effect of genotype of the incompatibility barrier in crossing *Pinus nigra* with *Pinus sylvestris*. Proc. of the 8th Congress of EUCARPIA, Madrid: 369-375.

VIDAKOVIĆ, M. (1983): Viability of seeds, obtained from back cross, as an indicator of the degree of incompatibility between *Pinus nigra* and *P. sylvestris*. Ann. Forest. **10/4**: 81-87.

VIDAKOVIĆ, M. (1986): Relation of self-fertilization and interspecific incompatibility by crossing *Pinus nigra* × *P. sylvestris*. Ann. Forest. **12/1**: 1-14.

VIDAKOVIĆ, M. & Ž. BORZAN (1991): The growth of some interspecific hybrid pine seedlings and their cuttings. Ann. Forest. **17/1**: 1-21.

YAZDANI, R. & J.E. NILSSON (1986): Cortical monoterpene variation in natural populations of *Pinus sylvestris* in Sweden. Scand. J. For. Res. **1**: 85-93.

YAZDANI, R., NILSSON, J.E. & T. ERICSSON (1985): Geographical variation in the relative proportion of monoterpenes in cortical oleoresin of *Pinus sylvestris* in Sweden. Silvae Genet. **34 (6)**: 201-208.

© Verlag Alexander Just: Dorfbeuern ... Download unter www.biologiezentrum.at

Addresses:

Dr. Marilena IDŽOJTIĆ
Department of Forest Genetics and Dendrology
Faculty of Forestry, University of Zagreb
Svetošimunska 25
HR-10000 Zagreb
Croatia
E-Mail: marilena.idzojtic@post.hinet.hr

Dr. Hartwig W. PFEIFHOFER
Department of Plant Physiology
University of Graz
Schubertstrasse 51
A-8010 Graz
E-Mail: hartwig.pfeifhofer@uni-graz.at

Table 1: Volatile compounds in needle oil of *Pinus sylvestris* (= sy), *P. nigra* (= ni), and their F_1 and F_2 hybrids (= F_1 nisy, F_2 nisy). Linear retention indices on the apolar column and quantification for all compounds are shown.

Peak	Compound	sy		ni		F_1 nisy		F_2 nisy	
		%	Ret. Index	%	Ret. Index	%	Ret. Index	%	Ret. Index
1	*Trans*-2-hexenal	0.12	834	0.1	835	0.13	833	0.11	832
2	Tricyclene	0.7	917	0.19	920	1.21	918	1.27	916
3	α-thujene	0.1	922	0.28	925	0.22	923	0.23	921
4	α-pinene	32.91	929	42.66	935	36.24	931	45.14	929
5	Camphene	3	940	1.13	943	5.34	941	5.5	939
6	Sabinene	0.33	963	traces		0.41	963	0.36	962
7	β-pinene	1.93	966	11.64	971	5.31	967	4.9	965
8	Myrcene	1.37	982	1.39	986	1.95	983	2.34	981
9	α-phellandrene	0.04	995	0.06	998	0.05	996	0.05	994
10	Δ-3-carene	8.86	1003	0.2	1005	5.41	1004	2.32	1002
11	α-terpinene	0.09	1007	0.06	1010	0.1	1008	0.07	1006
12	*p*-cymene	traces		0.02	1014	Traces		traces	
13	β-phellandrene + limonene	1	1018	3.66	1022	1.5	1018	2.18	1016
14	*Cis*-ocimene	0.04	1027	0.02	1030	0.03	1028	0.05	1027
15	*Trans*-β-ocimene	1.98	1038	1.03	1041	1.71	1038	0.96	1037
16	γ-terpinene	0.16	1047	0.05	1050	0.15	1047	0.1	1046
17	α-terpinolene	1.16	1076	0.61	1079	1.27	1077	0.98	1075

© Verlag Alexander Just: Dornbirn, Salzburg - Brüssel - Lindau - Zürich - Universitätsbibliothek Innsbruck - Forschungszentrum.at

18	Linalool	traces		0.06	1088	traces		traces	
19	α-campholene aldehyde	0		0.03	1107	traces		traces	
20	Camphor	traces		0.05	1122	0.01	1120	traces	
21	Borneol	traces		0.05	1148	0.02	1147	0.05	1145
22	Terpinen-4-ol	0.04	1159	0.05	1161	0.08	1159	0.05	1158
23	α-terpineol	traces		0.19	1173	0.05	1171	0.06	1170
24	methyl thymylether	traces		0.04	1218	0.02	1216	traces	
25	linalyl acetate	0.03	1240	0.22	1244	0.02	1242	0.05	1241
26	bornyl acetate	0.24	1267	0.81	1269	2.99	1268	3.84	1267
27	α-terpinyl acetate	0.2	1330	0.37	1333	0.25	1331	0.09	1330
28	bicycloelemene	0.07	1341	0.02	1343	0.04	1341	traces	
29	geranyl acetate	traces		0.01	1364	0.03	1364	traces	
30	α-copaene	0.19	1366	0.07	1368	0.1	1366	0.07	1366
31	β-bourbonene	0		0.23	1378	0.03	1375	0.04	1374
32	β-elemene	0.27	1380	0.02	1383	0.13	1380	0.15	1380
33	β-caryophyllene	4.05	1406	5.62	1411	4.66	1408	3.55	1406
34	β-cubebene	0.05	1415	0.03	1418	0.05	1416	0.03	1415
35	Aromadendrene	0.15	1425	0		0.09	1426	0.03	1425
36	α-humulene	0.68	1438	0.9	1442	0.85	1439	0.59	1438
37	sesquiterpene hydrocarbon (M 204)	0.3	1447	0.07	1450	0.07	1447	0.08	1447
38	γ-muurolene	0.49	1462	0		0		0	
39	germacrene D	3.45	1466	17.72	1474	11.6	1469	10.91	1467
40	α-muurolene	4.3	1481	0.26	1484	1.18	1482	0.96	1480
41	β-cadinene	0		0.03	1494	0.04	1491	0.03	1491
42	γ-cadinene	2.7	1497	0.25	1500	0.94	1498	0.68	1497
43	δ-cadinene	3.9	1508	0.48	1510	1.62	1508	1.16	1507
44	4,10-dimethyl-7-isopropyl (4,4,0)-bicyclo-1,4-decadiene	0.07	1515	0.01	1518	0.03	1515	0.03	1515
45	α-cadinene	0.17	1521	0.03	1524	0.39	1521	0.06	1521
46	*endo*-1-bourbonanol	9.86	1557	0.58	1558	3.01	1556	3.07	1556
47	caryophyllene oxide	0		0.1	1561	0.01	1563	0	
48	oxygenated sesquiterpene (M 222)	0.13	1605	0.02	1608	0.08	1605	0.04	1606
49	α-cadinol	2.07	1618	0.23	1621	0.81	1618	0.66	1618
50	sesquiterpene hydrocarbon (M 204)	0.24	1621	0.05	1624	0.12	1621	0.14	1621
51	T-muurolol	2.41	1630	0.27	1633	0.95	1630	0.86	1630
52	oxygenated sesquiterpene (M 220)	0		0.09	1662	0.02	1658	0	
53	13-epimanoyl oxyde	0.2	1967	2.25	1973	0.11	1968	0.04	1969
54	Thunbergol	traces		0.09	2031	traces		0.02	2028
Σ		90.1		94.4		91.4		93.9	

Figure 1: Components with more than 10 % of the total essential needle oil in at least one group (species or hybrid).

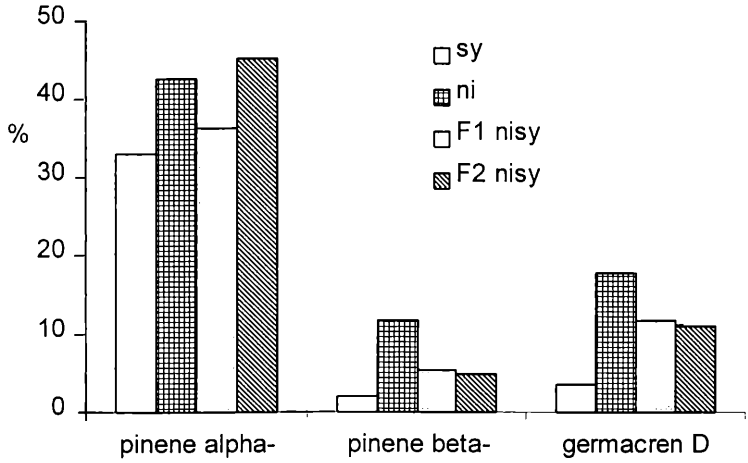


Figure 2: Components with more than 5 % of the total essential needle oil in at least one group (species or hybrid).

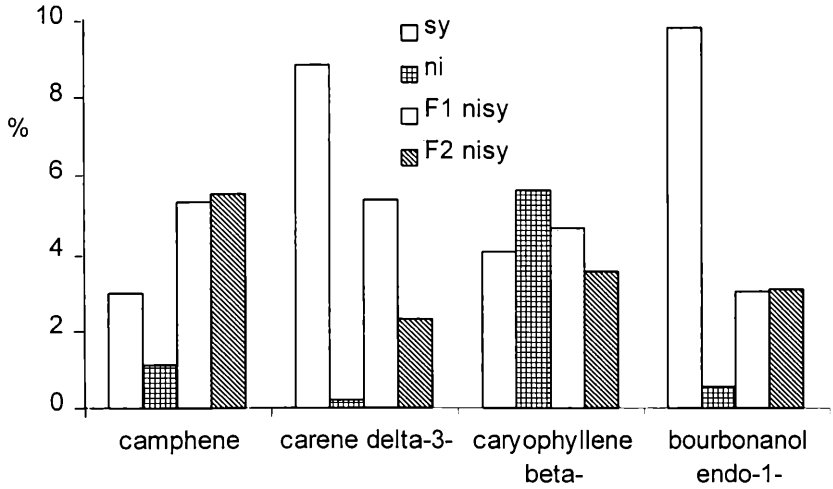


Figure 3: Components with more than 3 % of the total essential needle oil in at least one group (species or hybrid).

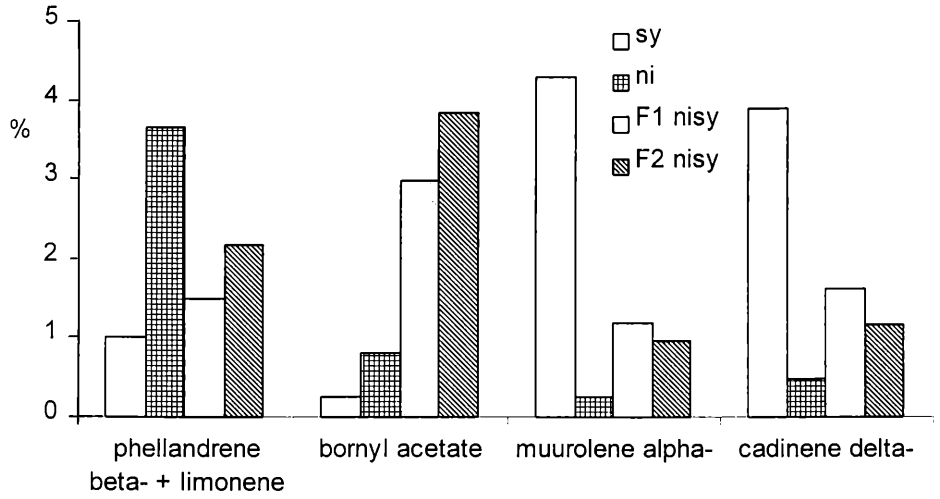


Figure 4: Components with more than 1% of the total essential needle oil in at least one group (species or hybrid). A = tricyclene, B = myrcene, C = *trans*-β-ocimene, D = α-terpinolene, E = γ-cadinene, F = α-cadinol, G = T-muurolol, H = 13-epimanoyl oxyde.

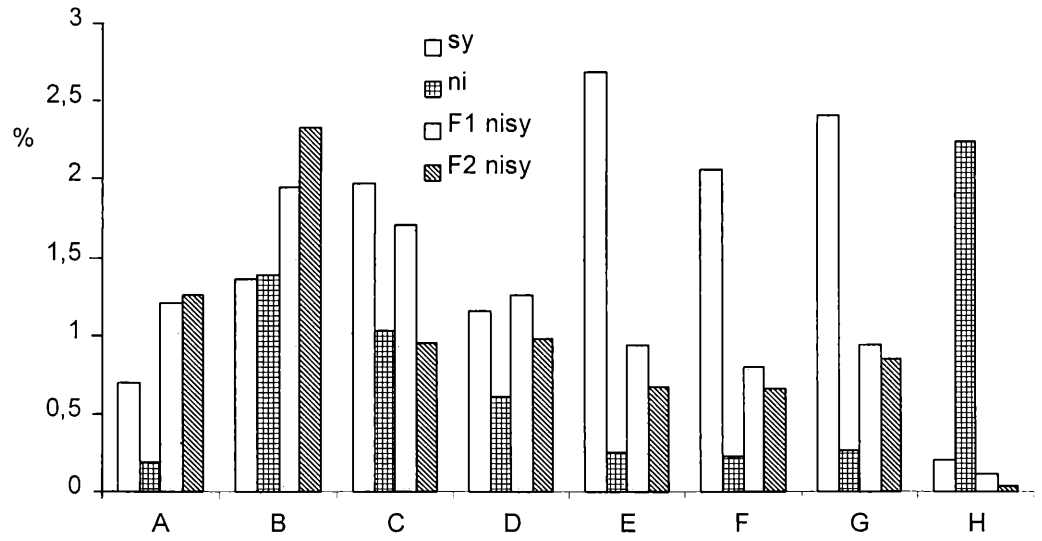
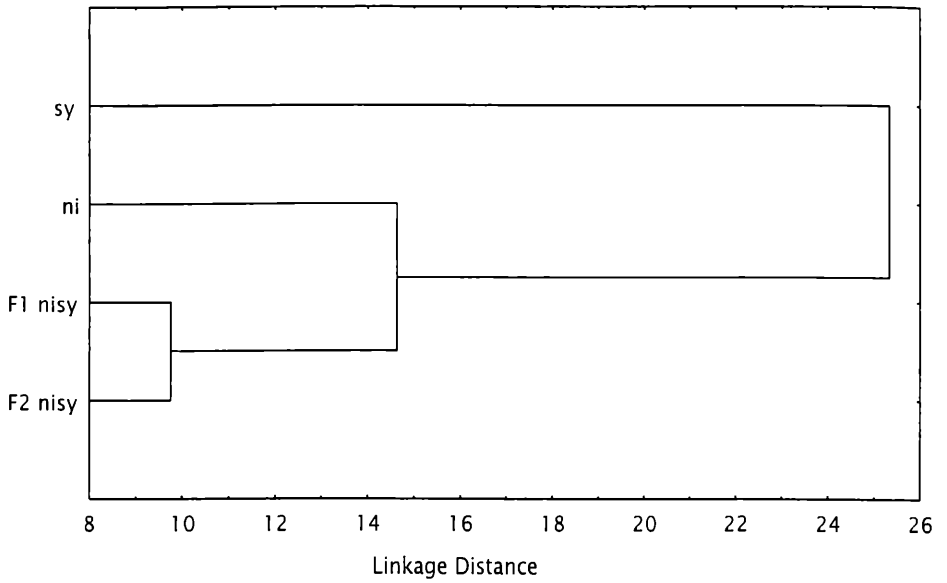


Figure 5: Horizontal hierarchical tree for four analyzed groups.



ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Sauteria-Schriftenreihe f. systematische Botanik, Floristik u. Geobotanik

Jahr/Year: 2001

Band/Volume: 11

Autor(en)/Author(s): Pfeifhofer Hartwig Wilfried, Idzojtic Marilena

Artikel/Article: Die Zusammensetzung der flüchtigen Nadelinhaltstoffe von Pinus nigra ARNOLD, P. sylvestris L. und ihrer Hybriden 367-382