

# 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

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**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<sub>1</sub> and F<sub>2</sub> hybrids (=F<sub>1</sub> nisy, F<sub>2</sub> 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<sub>1</sub> nisy 129, F<sub>2</sub> nisy 83). A total of 55 components was identified, of which 51 were unambiguously 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<sub>1</sub> hybrids, and 93.9% w/w sample of F<sub>2</sub> 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<sub>1</sub> nisy 64%, F<sub>2</sub> nisy 71%), followed by sesquiterpenes (ni 27%, sy 36%, F<sub>1</sub> nisy 27%, F<sub>2</sub> nisy 23%) and diterpenes (ni 2.34%, sy 0.2%, F<sub>1</sub> nisy 0.11%, F<sub>2</sub> nisy 0.16%). Cluster analysis shows that the hybrids F<sub>1</sub> and F<sub>2</sub> 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<sub>1</sub>- und F<sub>2</sub>-Hybriden (=F<sub>1</sub> nisy, F<sub>2</sub> nisy) wurde mittels Gaschromatographie (GC) und Gaschromatographie/Massenspektrometrie untersucht. Die Anzahl nachgewiesener Verbindungen schwankte je nach Art und Hybride (ni 141, sy 107, F<sub>1</sub> nisy 129, F<sub>2</sub> 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<sub>1</sub>-Hybriden und 93.9% des Nadelöls der F<sub>2</sub>-Hybriden aus. Mit Ausnahme des Aldehydes *trans*-2-Hexenal waren alle identifizierten Verbindungen Terpene. Monoterpene hatten den größten Anteil (ni 65%, sy 54%, F<sub>1</sub> nisy 64%, F<sub>2</sub> nisy 71%), gefolgt von Sesquiterpenen (ni 27%, sy 36%, F<sub>1</sub> nisy 27%, F<sub>2</sub> nisy 23%) und Diterpenen (ni 2.34%, sy 0.2%, F<sub>1</sub> nisy 0.11%, F<sub>2</sub> nisy 0.16%). Eine Clusteranalyse ergab, dass die F<sub>1</sub>- und F<sub>2</sub>-Hybriden sich am ähnlichsten sind und dass beide Hybridgruppen der Schwarzkiefer (*P. nigra*) ähnlicher sind als der Rotkiefer (*P. sylvestris*).

## Introduction

The F<sub>1</sub> and F<sub>2</sub> 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<sub>1</sub> hybrids (=F<sub>1</sub> 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<sub>1</sub> hybrids ((*P. nigra* × *P. sylvestris*) × (*P. nigra* × *P. sylvestris*)), in 1984 and 1985 the hybrids of F<sub>2</sub> generation were produced (=F<sub>2</sub> nisy). BORZAN & IDŽOJTIĆ (1996) and IDŽOJTIĆ (1996, 1997) reported about the morphology of needles in the F<sub>2</sub> 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<sub>1</sub>, F<sub>2</sub> 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<sub>1</sub> hybrids from 32 trees and of F<sub>2</sub> hybrids from 35 trees. For each species, i.e. hybrid, 14-25 g of needles were distilled in a Karlsruhe-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 × 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, Palo 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<sub>1</sub> nisy 129 and F<sub>2</sub> nisy 83). A total of 55 components was identified, of which 51 were unambiguously identified and 4 were partially characterized. Those 55 components are shown in Table 1.

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<sub>1</sub> hybrids and 93.9% w/w sample of F<sub>2</sub> 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  $\alpha$ -pinene, 32.91% and  $\Delta$ -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%;  $\alpha$ -muurolene, 4.3%;  $\beta$ -caryophyllene, 4.05%;  $\delta$ -cadinene, 3.9%, germacrene D, 3.45% and  $\gamma$ -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  $\Delta$ -3-carene (CHALCHAT et al., 1985; ORAV et al., 1996). The Scots pine trees autochthonous in Croatia, whose needles were analyzed in this paper, seem to belong to the chemotype with a low  $\Delta$ -3-carene content. The amount of  $\Delta$ -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  $\alpha$ -pinene (42%) and  $\beta$ -phellandrene + limonene (5.5%), than in this paper. The amount of  $\Delta$ -3-carene was also high (20.6%), indicating that the sample originated from a Scots pine of the high  $\Delta$ -3-carene chemotype. However, the provenance of the samples was not reported by the authors.

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  $\alpha$ -pinene, 42.66%,  $\beta$ -pinene, 11.64% and  $\beta$ -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  $\beta$ -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  $\alpha$ -pinene. Needle volatiles analyzed by CHALCHAT & GORUNOVIC (1995a) had a higher amount of  $\alpha$ -pinene (66.5%) and  $\beta$ -phellandrene + limonene (6%), and a lower amount of  $\beta$ -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  $\alpha$ -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<sub>1</sub> 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  $\alpha$ -pinene, 36.24%;  $\Delta$ -3-carene, 5.41%; camphene 5.34%;  $\beta$ -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%;  $\beta$ -caryophyllene, 4.66%; *endo*-1-bourbonanol 3.01%;  $\delta$ -cadinene 1.62% and  $\alpha$ -muurolene 1.18% (Figure 1, 2, 3). Two diterpenes were identified, 13-epimanoyl oxyde, 0.11% (Figure 4) and thunbergol, in traces.

## **F<sub>2</sub> 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  $\alpha$ -pinene, 45.14%; camphene 5.5%;  $\beta$ -pinene, 4.9%; bornyl acetate 3.84%; myrcene 2.34%;  $\Delta$ -3-carene, 2.32% and  $\beta$ -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%;  $\beta$ -caryophyllene, 3.55%; *endo*-1-bourbonanol 3.07% and  $\delta$ -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%),  $\Delta$ -3-carene (sy 8.86%, ni 0.2%),  $\alpha$ -muurolene (sy 4.3%, ni 0.26%),  $\gamma$ -cadinene (sy 2.7%, ni 0.25%), T-muurolol (sy 2.41%, ni 0.27%) and  $\alpha$ -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%),  $\beta$ -pinene (sy 1.93%, ni 11.64%),  $\beta$ -phellandrene + limonene (sy 1%, ni 3.66%) and 13-epimanoyl oxide (sy 0.2%, ni 2.25%).

The amount of most components in the F<sub>1</sub> and F<sub>2</sub> hybrids is intermediate as compared to the parental species.

In all four analyzed groups (species and hybrids),  $\alpha$ -pinene had the highest concentration (sy 32.9%, ni 42.7%, F<sub>1</sub> nisy 36.24% and F<sub>2</sub> nisy 45.14%). The amount of this monoterpene in the F<sub>1</sub> hybrids was intermediate compared to the parental species, while in the F<sub>2</sub> hybrids it was higher than in both parental species.

The amount of  $\beta$ -pinene, germacrene D,  $\Delta$ -3-carene, *endo*-1-bourbonanol,  $\beta$ -phellandrene + limonene,  $\alpha$ -muurolene,  $\gamma$ -cadinene,  $\alpha$ -cadinol and T-muurolol in the F<sub>1</sub> and F<sub>2</sub> hybrids is intermediate in relation to the parental species.

The amount of *trans*- $\beta$ -ocymene and  $\beta$ -caryophyllene is intermediate in F<sub>1</sub> hybrids and lower in F<sub>2</sub> hybrids than in both parental species. F<sub>1</sub> hybrids contain more  $\alpha$ -terpinolene than both parental species, while its content is intermediate in F<sub>2</sub> hybrids.

Needle oils of F<sub>1</sub> and F<sub>2</sub> hybrids have a higher concentration of the diterpene 13-epimanoyl oxide 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<sub>1</sub> and F<sub>2</sub> 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<sub>1</sub> and F<sub>2</sub> hybrids are more similar to the European black pine, which is the female parent of F<sub>1</sub> hybrids, than to the Scots pine.



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<sub>1</sub> nisy and 83 for F<sub>2</sub> nisy. A total of 55 components was identified, of which 51 were unambiguously 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<sub>1</sub> hybrids and 93.9% w/w sample of F<sub>2</sub> 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<sub>1</sub> nisy 64%, F<sub>2</sub> nisy 71%), followed by sesquiterpenes (ni 27%, sy 36%, F<sub>1</sub> nisy 27%, F<sub>2</sub> nisy 23%) and diterpenes (ni 2.34%, sy 0.2%, F<sub>1</sub> nisy 0.11%, F<sub>2</sub> 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<sub>1</sub> and F<sub>2</sub> hybrids is intermediate as compared to the parental species.

Cluster analysis shows that the hybrids F<sub>1</sub> and F<sub>2</sub> are the most similar, and that both hybrid groups are more similar to European black pine (*P. nigra*) than to Scots pine (*P. sylvestris*).

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Table 1: Volatile compounds in needle oil of *Pinus sylvestris* (= sy), *P. nigra* (= ni), and their F<sub>1</sub> and F<sub>2</sub> hybrids (= F<sub>1</sub> nisy, F<sub>2</sub> nisy). Linear retention indices on the apolar column and quantification for all compounds are shown.

Peak	Compound	sy		ni		F <sub>1</sub> nisy		F <sub>2</sub> nisy	
		%	Ret. Index	%	Ret. Index	%	Ret. Index	%	Ret. Index
1	<i>Trans</i> -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	$\alpha$ -thujene	0.1	922	0.28	925	0.22	923	0.23	921
4	$\alpha$ -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	$\beta$ -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	$\alpha$ -phellandrene	0.04	995	0.06	998	0.05	996	0.05	994
10	$\Delta$ -3-carene	8.86	1003	0.2	1005	5.41	1004	2.32	1002
11	$\alpha$ -terpinene	0.09	1007	0.06	1010	0.1	1008	0.07	1006
12	<i>p</i> -cymene	traces		0.02	1014	Traces		traces	
13	$\beta$ -phellandrene + limonene	1	1018	3.66	1022	1.5	1018	2.18	1016
14	<i>Cis</i> -ocimene	0.04	1027	0.02	1030	0.03	1028	0.05	1027
15	<i>Trans</i> - $\beta$ -ocimene	1.98	1038	1.03	1041	1.71	1038	0.96	1037
16	$\gamma$ -terpinene	0.16	1047	0.05	1050	0.15	1047	0.1	1046
17	$\alpha$ -terpinolene	1.16	1076	0.61	1079	1.27	1077	0.98	1075

18	Linalool	traces		0.06	1088	traces		traces	
19	$\alpha$ -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	$\alpha$ -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	$\alpha$ -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	$\alpha$ -copaene	0.19	1366	0.07	1368	0.1	1366	0.07	1366
31	$\beta$ -bourbonene	0		0.23	1378	0.03	1375	0.04	1374
32	$\beta$ -elemene	0.27	1380	0.02	1383	0.13	1380	0.15	1380
33	$\beta$ -caryophyllene	4.05	1406	5.62	1411	4.66	1408	3.55	1406
34	$\beta$ -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	$\alpha$ -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	$\gamma$ -muurolene	0.49	1462	0		0		0	
39	germacrene D	3.45	1466	17.72	1474	11.6	1469	10.91	1467
40	$\alpha$ -muurolene	4.3	1481	0.26	1484	1.18	1482	0.96	1480
41	$\beta$ -cadinene	0		0.03	1494	0.04	1491	0.03	1491
42	$\gamma$ -cadinene	2.7	1497	0.25	1500	0.94	1498	0.68	1497
43	$\delta$ -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	$\alpha$ -cadinene	0.17	1521	0.03	1524	0.39	1521	0.06	1521
46	<i>endo</i> -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	$\alpha$ -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
$\Sigma$		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).

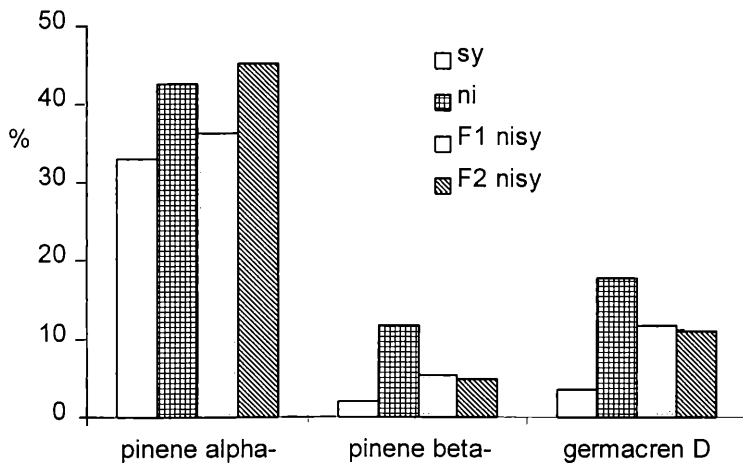


Figure 2: Components with more than 5 % of the total essential needle oil in at least one group (species or hybrid).

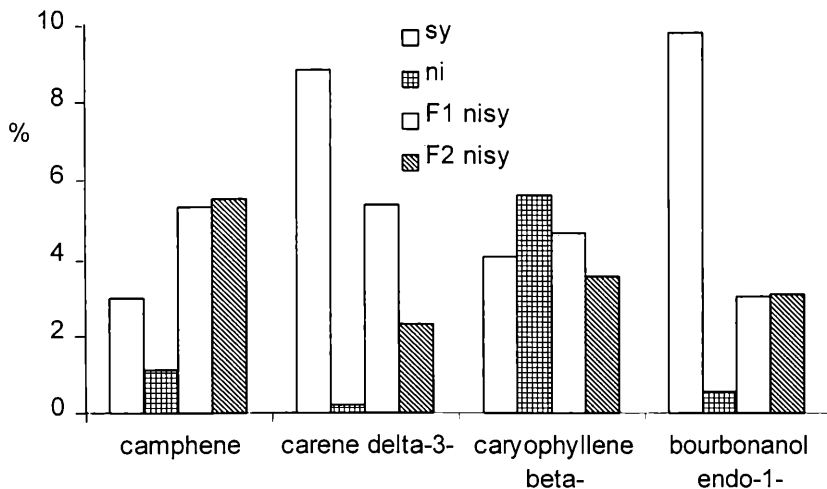


Figure 3: Components with more than 3% of the total essential needle oil in at least one group (species or hybrid).

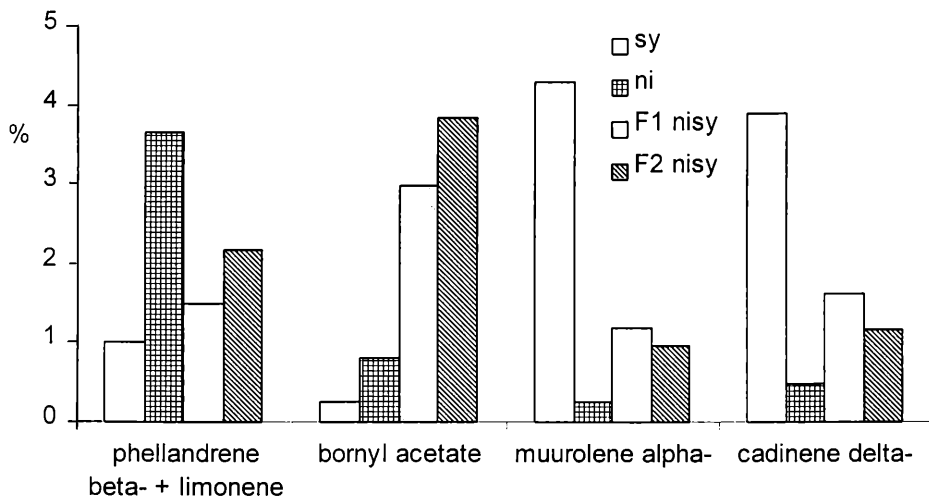


Figure 4: Components with more than 1% of the total essential needle oil in at least one group (species or hybrid). A = tricyclene, B = myrcene, C = *trans*- $\beta$ -ocimene, D =  $\alpha$ -terpinolene, E =  $\gamma$ -cadinene, F =  $\alpha$ -cadinol, G = T-muurolol, H = 13-epimanoyl oxyde.

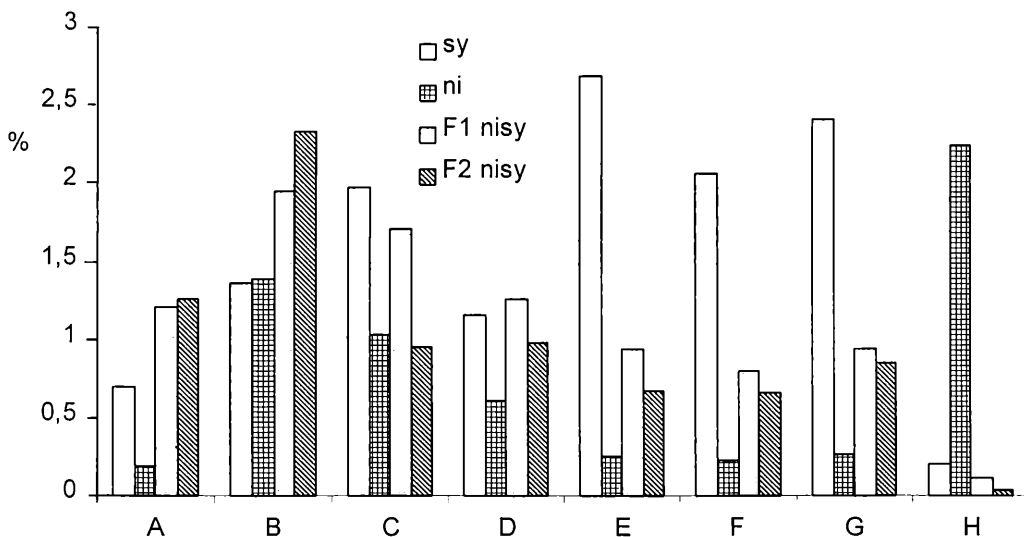


Figure 5: Horizontal hierarchical tree for four analyzed groups.

