

The Needle Volatile Composition of *Pinus nigra* J. F. Arnold, *P. sylvestris* L., *P. densiflora* Siebold et Zucc. and *P. thunbergiana* Franco Trispecies Hybrids

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Abstract

The needle volatile composition of two trispecies hard pine hybrids produced by the controlled hybridization and their parents was researched with gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) in order to confirm their hybridity. The analysed trispecies hybrids were: (*Pinus nigra* × *P. sylvestris*) × *P. densiflora* (= *nisyde*) and (*P. nigra* × *P. sylvestris*) × *P. thunbergiana* (= *nisyth*). A total of 59 compounds was identified. All identified compounds were terpenes, except one.

The trispecies hybrids showed the same qualitative pattern of the needle volatile composition as their parental species. However, there were quantitative differences in several major terpenes. According to the needle volatile composition the hybrids *nisyde* were more similar to the female parent, whereas the hybrids *nisyth* were more similar to the male parent. The hybrids *nisyth* had a considerably higher content of β-pinene (specific component of *P. thunbergiana*) than the other analyzed groups. The content of thunbergol (specific component of *P. densiflora*) in the hybrids *nisyde* was not considerably different in relation to the other groups. The intermediary quality of the hybrids *nisyde* and *nisyth* for most components in relation to their parents, as well as the previous morphometric analysis, confirms their hybrid character.

Key words: trispecies hybrids, (*Pinus nigra* × *P. sylvestris*) × *P. densiflora*, (*Pinus nigra* × *P. sylvestris*) × *P. thunbergiana*, needle volatiles, GC, GC/MS.

Introduction

The trispecies hybrids (*Pinus nigra* J. F. Arnold × *P. sylvestris* L.) × *P. densiflora* Siebold et Zucc. and (*Pinus nigra* J. F. Arnold × *P. sylvestris* L.) × *P. thunbergiana* Franco were produced by controlled hybridization at the Faculty of Forestry, University of Zagreb. European black pine (*Pinus nigra*), Scots pine (*P. sylvestris*), Japanese red pine (*P. densiflora*) and Japanese black pine (*P. thunbergiana*) were used to create different hybrid families by various crossing combinations, including production of F₁ hybrids, back-crossed and reciprocal hybrids, F₂ hybrids, and trispecies hybrids.

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The trispecies hybrids were produced in 1985 and planted on the experimental plot at Arboretum Lisičine. Due to similarities in habit, growth and morphological traits among trispecies hybrids and their parents, hybrid identification and evaluation has been difficult. Identification of these hybrids was carried out by discriminant analyses using nineteen needle and shoot internal and external morphological traits (BORZAN and IDŽOJTIĆ, 2002).

The chemical composition of various pine species volatiles have been the subject of numerous studies (e.g. CHALCHAT *et al.*, 1985; KUBECZKA and SCHULTZE, 1987; SCHILLER and GRUNWALD, 1987; ROUSSIS *et al.*, 1995; KOUKOS *et al.*, 2000; GOMES DA SILVA *et al.*, 2001; KUROSE *et al.*, 2007). Since the biosynthesis of the various terpenes is under genetic control (CROTEAU *et al.*, 2000), analysis of the terpene composition of needle oils is a useful tool in studies of biosystematics and chemotaxonomy. In this study the needle volatile composition of trispecies hybrids in relation to their parents has been researched in order to explore the utility of terpenes in hybrid identification (their differentiation from the parents) as well as confirmation of hybridity.

The basic needle volatile compounds of pines are terpenes. GALLIS and PANETSOS (1997) researched the possibility to distinguish *Pinus brutia* Ten., *P. halepensis* Mill. and their F₁, F₂ 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.

The composition of needle volatiles of *P. nigra*, *P. sylvestris* and their F₁ and F₂ hybrids was researched by IDŽOJTIĆ and PFEIFHOFER (2001). The F₁ and F₂ hybrids were the most similar, and both hybrid groups were more similar to European black pine than to Scots pine.

The hybrid character of F₁ hybrids *nisy* was confirmed by the morphometrical research (IDŽOJTIĆ, 1997; BORZAN *et al.*, 2002) as well as by the analysis of the needle volatile composition (IDŽOJTIĆ, 2001; IDŽOJTIĆ and PFEIFHOFER, 2001; IDŽOJTIĆ *et al.*, 2005).

Abbreviations for species and hybrid names have been used as first two letter of the species name or the hybrid combination: 1. (*Pinus nigra* × *P. sylvestris*) × *P. densiflora* (= *nisyde*); 2. (*Pinus nigra* × *P. sylvestris*) × *P. thunbergiana* (= *nisyth*); 3. *P. nigra* × *P. sylvestris* (= *nisy*); 4. *P. densiflora* (= *de*); 5. *P. thunbergiana* (= *th*); 6. *P. nigra* (= *ni*); 7. *P. sylvestris* (= *sy*).

Materials and Methods

Plant Material and Sample Preparation

The sampled trees (hybrids and species) were located in three experimental plots in Croatia. The trees of four pure species were control trees in those plots, grafted or produced by open pollination of the hybrids' parental trees. The pure species trees were of similar age to the sampled hybrid trees. Needles were taken from fully developed one-year old shoots. For each type (hybrid or species) two shoots from each tree were taken in the same vegetation period, from as many different trees as possible, however, the number of samples per type varied according to plot size and survival at the different locations. Samples of F_1 hybrids *nisy* were taken from 32 trees, of Japanese red pine from 40 trees, of Japanese black pine from 9 trees, of European black pine from 41 trees, of Scots pine from 29 trees, of trispecies hybrid *nisyde* from 21 and of *nisyth* from 8 trees. Samples were devoid of illness and insect attacks. For each species, i.e. hybrid, fresh needles of all sampled trees were mixed in equal mass proportion and then distilled in a Karl-sruher-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 μ m) 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 μ m) capillary column (J and 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 μ m; J and 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 and 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.

The analysis was performed at the Institute for Plant Sciences of the University of Graz, Austria.

Cluster Analysis

To determine the degree of similarities between analysed trispecies hybrids and their parents, the area percent values were subjected to cluster analysis (tree clustering algorithm, Euclidean distance, single linkage method).

Data were processed using the statistical software StatSoft.Inc (2001).

Results

The number of detected compounds varied with hybrids and species (*nisy* 129, *de* 122, *th* 87, *ni* 141, *sy* 107, *nisyde* 96, *nisyth* 72). A total of 59 compounds were identified, 53 of which were unambiguously identified and 6 were partially characterized (Table 1). All identified compounds were terpenes, except trans-2-hexenal. They accounted for 98.3% w/w sample of F_1 hybrids *nisy*, 95.9% of *P. densiflora*, 98.4% of *P. thunbergiana*, 92.9% of *P. nigra*, 97.7% of *P. sylvestris*, 97.9% of trispecies hybrid *nisyde* and 98.9% of trispecies hybrid *nisyth*.

Trispecies Hybrids (P. nigra x P. sylvestris) x P. densiflora

The main substance class were monoterpenes (N = 26), comprising 64.1% of the total needle volatile content. The major compounds were α -pinene, 31.7%; bornyl acetate, 7.4%; camphene 5.3%; α -terpinolene, 4.1%; β -pinene, 4%; myrcene, 3.7% and β -phellandrene + limonene, 3.2% (Table 1). The content of those compounds in the trispecies hybrids *nisyde* (Figure 1) was intermediate in relation to the parental species (α -terpinolene, myrcene and β -phellandrene + limonene), higher than in both parental species, but more similar to the female parent *nisy* (α -pinene, bornyl acetate and camphene) or lower than in both parental species, but also more similar to the female parent *nisy* (β -pinene).

The second main substance class were sesquiterpenes (N = 27), 22 of which were identified (32.8%) and 5 partially characterized (0.4%). The major compounds were

Table 1. – Needle volatile composition of trispecies hybrids (*Pinus nigra* × *P. sylvestris*) × *P. densiflora* (= *nisy* × *de*) and (*Pinus nigra* × *P. sylvestris*) × *P. thunbergiana* (= *nisy* × *th*), F₁ hybrid *P. nigra* × *P. sylvestris* (= *nisy*), *P. densiflora* (= *de*), *P. thunbergiana* (= *th*), *P. nigra* (= *ni*) and *P. sylvestris* (= *sy*). Traces = (<0.05%).

Peak	Compound	Ret. Index	% nisy × de nisy × th nisy de th ni sy						
			1	<i>trans</i> -2-hexenal	820	0.2	0.1	0	0.1
2	tricyclene	921	1.6	0.8	1.2	1.1	0.5	0.2	0.7
3	<i>α</i> -thujene	924	0.2	0.1	0.3	traces	traces	0.3	0.1
4	<i>α</i> -pinene	936	31.7	26.3	28.9	17.6	18.5	42.7	24.7
5	camphene	946	5.3	2.9	5.2	3.5	2.0	1.1	2.8
6	sabinene	967	0.4	0.4	0.4	0.5	0.3	traces	0.3
7	β -pinene	974	4.0	18.3	5.2	5.6	29.1	11.6	1.8
8	myrcene	983	3.7	3.3	2.0	8.4	5.1	1.4	1.3
9	<i>α</i> -phellandrene	997	0.1	0.1	0.1	0.2	0.1	0.1	0
10	Δ -3-carene	1006	0.2	traces	5.2	0.1	0.1	0.2	7.5
11	<i>α</i> -terpinene	1009	0.1	traces	0.1	0.1	0.1	0.1	0.1
12	β -phellandrene + limonene	1024	3.2	8.2	1.7	11.3	11.1	3.7	1
13	<i>cis</i> - β -ocimene	1025	0.1	0.1	0.1	0	0	0	0.1
14	<i>trans</i> - β -ocimene	1037	1.2	0.8	1.8	traces	0.1	1	1.8
15	γ -terpinene	1050	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16	<i>α</i> -terpinolene	1081	4.1	1.9	1.3	4.7	2.8	0.6	1.1
17	borneol	1152	traces	traces	0	0.1	0.1	0.1	traces
18	terpinen-4-ol	1164	0.1	0.1	0.1	0.1	0.1	0.1	0
19	(-)-myrtenal	1171	traces	0	0	0.1	0	0	0
20	<i>α</i> -terpinol	1174	0.1	0.2	0.1	0.1	0.7	0.2	traces
21	methyl thymylether	1214	traces	0.2	traces	0.3	0.3	0	traces
22	bornyl acetate	1273	7.4	4.7	5.1	4.7	3.1	0.8	0.5
23	<i>α</i> -terpinenyl acetate	1333	traces	0	0.4	0	0	0.4	0.3
24	δ -clmene	1338	0.3	0.4	0.4	0.4	0.2	0.1	0.9
25	<i>α</i> -cubebene	1352	traces	traces	0.1	traces	traces	0	0.1
26	geranyl acetate	1358	0.1	0	traces	0.4	0.4	0	traces
27	methyl eugenol	1369	0.3	0	traces	0.2	traces	0	0
28	<i>α</i> -copaene	1380	0.1	0.1	0.1	0.1	0.1	0.1	0.3
29	β -clmene	1391	1.2	1.2	1.1	1.2	0.5	0	2.8
30	longifolene	1413	traces	traces	0	0.1	0.1	0	0
31	β -caryophyllene	1427	6.3	5.5	6.8	7.9	4.7	5.6	5.2
32	β -cubebene	1433	0.2	0.2	0.4	0.3	0.2	0	0.2
33	<i>trans</i> - <i>α</i> -bergamotene	1435	traces	0	0	0	0.1	0	0
34	aromadendrene	1444	0.1	traces	0.1	0.1	traces	0	0.2
35	sesquiterpene hydrocarbon (M 204)	1447	0.1	0.1	0.2	0.2	0.1	0	0.1
36	sesquiterpene hydrocarbon (M 204)	1452	traces	0	traces	traces	0.1	0	0.1
37	<i>α</i> -humulene	1458	1.2	1.0	1.1	1.6	0.8	0.9	1
38	sesquiterpene hydrocarbon (M 204)	1464	0.1	0.1	0.1	0.1	0.1	0.1	0.1
39	sesquiterpene hydrocarbon (M 204)	1471	0.1	traces	0.1	0.1	0	0.5	0.1
40	γ -muurolene	1476	0.5	0.4	0.7	0.1	0.5	0.1	0.7
41	germaerene D	1486	10.0	9.2	12.8	8.5	9.2	17.7	3.3
42	β -selinene	1490	0.3	0.3	0.4	0.3	0.3	0.1	0.8
43	<i>α</i> -muurolene	1498	1.9	1.1	1.6	1.1	0.3	0.3	4.5
44	bicyclogermaerene	1498	0	0	0	0.9	0.9	0	0
45	sesquiterpene hydrocarbon (M 204)	1502	0.1	0.1	0.1	0.1	0.1	0.2	0
46	β -cadinene	1504	0.1	traces	0.1	0.1	0.1	0	0
47	γ -cadinene	1513	1.0	0.9	1.4	0.8	0.6	0.3	3.7
48	δ -cadinene	1520	1.7	1.4	2.2	1.2	1.0	0.5	5.1
49	4,10-dimethyl-7-isopropylbicyclo(4,4,0)-1,4-decadiene	1530	0.1	traces	0.1	traces	traces	0	0.2
50	<i>α</i> -cadinene	1535	0.1	0.1	0.1	0.1	0.1	0	0.2
51	<i>endo</i> -1-bourbonanol	1574	4.8	4.6	5.6	3.2	1.1	0.6	16.7
52	(-)-caryophyllene oxide	1580	0.1	0.1	0.1	0.2	0.1	0.1	0.1
53	<i>α</i> -cadinol	1634	1.4	1.2	1.5	0.9	0.5	0.2	3.4
54	<i>epi</i> - <i>α</i> -muurolol	1646	1.7	1.4	1.7	1.1	0.6	0.1	3.7
55	oxygenated sesquiterpene (M 222)	1675	0	0	0	0.1	0	0.2	0
56	farnesol	1702	0	0	0	0	0.1	0.2	0
57	<i>trans,trans</i> -farnesyl acetate	1815	traces	traces	0	0.1	0	0.1	0
58	sandaracopimaradiene	1978	traces	0	traces	0.2	traces	0	0
59	thunbergol	2057	0.1	1.1	0	5.4	1.2	0.1	traces
Σ			97.9	98.9	98.3	95.9	98.4	92.9	97.7

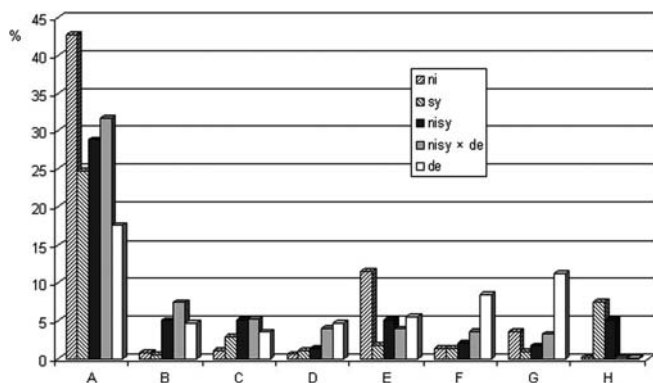


Figure 1. – Relative monoterpene content in *P. nigra* (ni), *P. sylvestris* (sy), F₁ hybrid (nisy), trispecies hybrid (nisy × de) and *P. densiflora* (de). A = α-pinene, B = bornyl acetate, C = camphene, D = α-terpinolene, E = β-pinene, F = myrcene, G = β-phellandrene + limonene, H = Δ-3-carene.

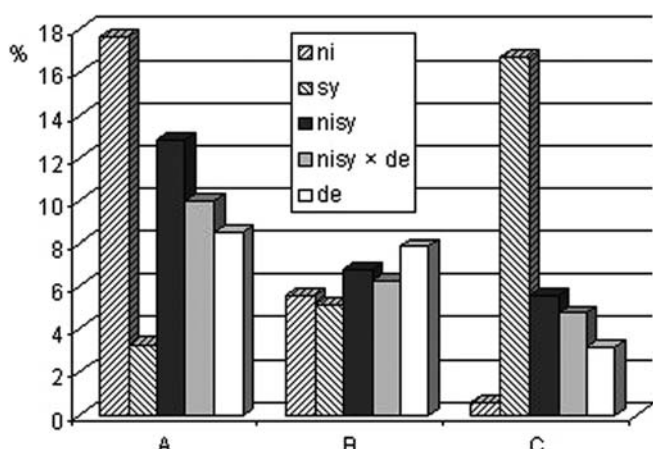


Figure 2. – Relative sesquiterpene content in *P. nigra* (ni), *P. sylvestris* (sy), F₁ hybrid (nisy), trispecies hybrid (nisy × de) and *P. densiflora* (de). A = germacrene D, B = β-caryophyllene, C = *endo*-1-bourbonanol.

germacrene D, 10%; β-caryophyllene, 6.3% and *endo*-1-bourbonanol 4.8%. The content of those sesquiterpenes (Figure 2) was intermediate in relation to the parental species (germacrene D and *endo*-1-bourbonanol) or lower than in both parents, but more similar to the female parent *nisy* (β-caryophyllene).

Trispecies Hybrids (*P. nigra* × *P. sylvestris*) × *P. thunbergiana*

The largest number of the identified compounds were monoterpenes (N = 22). They participated in the total volatile content with 68.9%. Monoterpenes with the highest proportion in the needle volatile content (Table 1) were α-pinene, 26.3%; β-pinene, 18.3%; β-phellandrene + limonene, 8.2%; bornyl acetate, 4.7%; myrcene, 3.3% and camphene, 2.9%. The content of these compounds in the trispecies hybrids *nisyth* was intermediate in relation to the parental species (Figure 3).

There were 25 sesquiterpenes (29.0%), 21 of which were identified (28.7%) and 4 partly described (0.3%). The major compounds were germacrene D, 9.2%; β-caryophyllene, 5.5% and *endo*-1-bourbonanol, 4.6%

(Table 1). According to the contents of β-caryophyllene and *endo*-1-bourbonanol, the trispecies hybrids *nisyth* were intermediary between their parents. The content of germacrene D in the trispecies hybrids was the same as in the male parent (Figure 4).

Table 1 shows also the needle volatile composition of *P. nigra* and *P. sylvestris*, the parental species of the trispecies hybrids female parent, *nisy*. The amount of the major compounds (Figure 1–4), α-pinene, β-pinene, Δ-3-carene, β-phellandrene + limonene, germacrene D and *endo*-1-bourbonanol in the F₁ hybrids was intermediate as compared to the parental species. F₁ hybrids contained more camphene, myrcene, α-terpinolene, bornyl acetate and β-caryophyllene than both parental species.

In Figure 5, the horizontal hierarchical tree is given for all analysed groups of hybrids and species together. According to the composition of needle volatiles, the most similar were trispecies hybrids *nisyde* and their female parent *nisy* (linkage distance 7.9). Then followed the trispecies hybrids *nisyth* and their male parent *th* (linkage distance 14.5). Those clusters were linked at

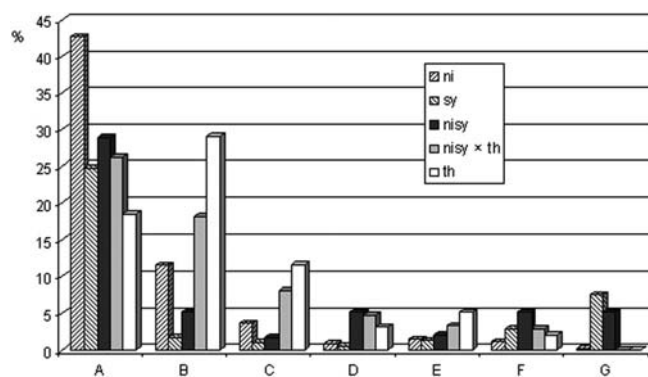


Figure 3. – Relative monoterpene content in *P. nigra* (ni), *P. sylvestris* (sy), F₁ hybrid (nisy), trispecies hybrid (nisy × th) and *P. thunbergiana* (th). A = α-pinene, B = β-pinene, C = β-phellandrene + limonene, D = bornyl acetate, E = myrcene, F = camphene, G = Δ-3-carene.

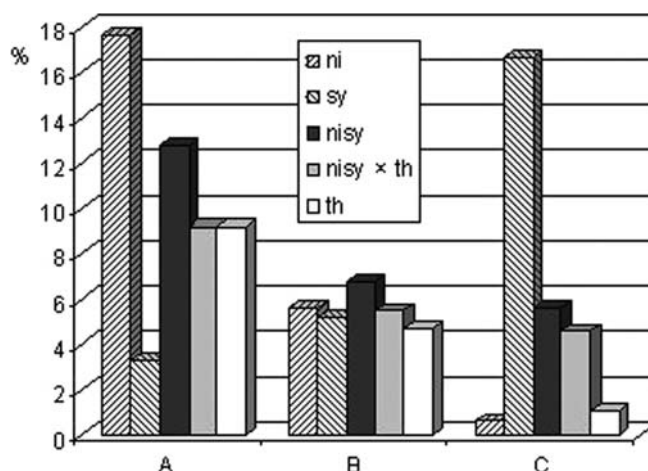


Figure 4. – Relative sesquiterpene content in *P. nigra* (ni), *P. sylvestris* (sy), F₁ hybrid (nisy), trispecies hybrid (nisy × th) and *P. thunbergiana* (th). A = germacrene D, B = β-caryophyllene, C = *endo*-1-bourbonanol.

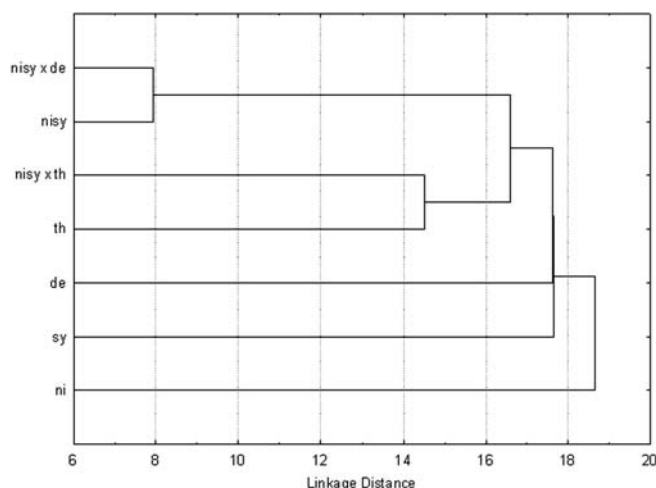


Figure 5. – Horizontal hierarchical tree for seven analysed groups.

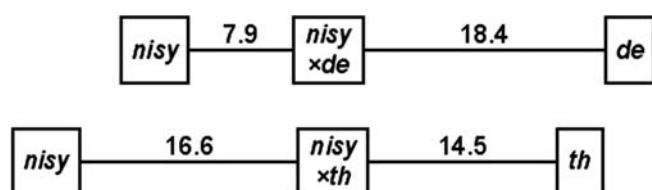


Figure 6. – Linkage distances between trispecies hybrids and their parents.

the distance of 16.6, and then at the distance of 17.6 *de* was joined, at the distance 17.7 *sy* was joined and finally *ni* was joined at the distance 18.7.

Figure 6 shows the linkage distances of trispecies hybrids *nisyde* and *nisyth* from their female parent, F_1 hybrid *nisy* as well as from the male parents, *P. densiflora* and *P. thunbergiana* respectively.

Discussion and Conclusion

The analysed trispecies hard pine hybrids *nisyde* and *nisyth* showed the same qualitative pattern of the needle volatile composition as their parental species. However, there were quantitative differences in several major terpenes between them. These results are consistent with the findings of GALLIS and PANETSOS (1997) for the cortical oleoresin terpene composition in hybrids *P. brutia* × *P. halepensis*.

In six of seven analysed groups (hybrids and species), α -pinene had the highest proportion in the total needle volatile content (*nisyde* 31.7%, *nisyth* 26.3%, *nisy* 28.9%, *de* 17.6%, *ni* 42.7, *sy* 24.7). Only in the needle volatiles of *P. thunbergiana* was the higher proportion of β -pinene (29.1%) than α -pinene (18.5%). Every trispecies hybrid parental species (*nisy*, *de* and *th*) had some needle volatile compounds with much higher proportions than in the volatiles of other analysed species. The compound specific of the female parent *nisy* was Δ -3-carene (*nisy* 5.2%, *de* 0.1%, *th* 0.1%). Thunbergol was specific compound of the Japanese red pine (*de* 5.4%, *th* 1.2%, *nisy* 0%), while β -pinene had the largest

proportion in the Japanese black pine (*th* 29.1%, *de* 5.6%, *nisy* 5.2%).

According to the content of Δ -3-carene (the specific component of the female parent, F_1 hybrid *nisy*) trispecies hybrids *nisyde* and *nisyth* were closer to the male parents (*nisyde* 0.2%, *nisyth* traces, *nisy* 5.2%, *de* 0.1%, *th* 0.1%). The content of thunbergol (specific component of the male parent *P. densiflora*) in the hybrids *nisyde* was not considerably higher in relation to the female parent (*nisyde* 0.1%, *nisy* 0%, *de* 5.4%). In view of the content of β -pinene, the specific component of *P. thunbergiana*, the trispecies hybrids *nisyth* were intermediary to the parental species and that content was considerably higher than in the other analyzed groups (*nisyth* 18.3%, *nisy* 5.2%, *th* 29.1%, *nisyde* 4%, *de* 5.6%).

Apart from the mentioned specific components for the distinction of hybrids from the parental species, i.e. the identification of the hybrids, other components can be used, which have a considerably different content in the parental species, and in the hybrids its content is intermediary in relation to the parental species. For the hybrids *nisyde* these components were: myrcene (*nisyde* 3.7%, *nisy* 2.0%, *de* 8.4%), α -terpinolene (*nisyde* 4.1%, *nisy* 1.3%, *de* 4.7%), germacrene D (*nisyde* 10.0%, *nisy* 12.8%, *de* 8.5%) and *endo*-1-bourbonanol (*nisyde* 4.8%, *nisy* 5.6%, *de* 3.2%). For the hybrids *nisyth* these were: camphene (*nisyth* 2.9%, *nisy* 5.2%, *th* 2.0%), myrcene (*nisyth* 3.3%, *nisy* 2.0%, *th* 5.1%), β -phellandrene + limonene (*nisyth* 8.2%, *nisy* 1.7%, *th* 11.1%), bornyl acetate (*nisyth* 4.7%, *nisy* 5.1%, *th* 3.1%), β -caryophyllene (*nisyth* 5.5%, *nisy* 6.8%, *th* 4.7%) and *endo*-1-bourbonanol (*nisyth* 4.6%, *nisy* 5.6%, *th* 1.1%).

Differences between the hybrid and the mean of the parents (as presented in Figures 1–4) could be interpreted as a measure of heterosis. The results suggest that most volatiles act additively, but a few are exceptions. Table 2 shows heterosis (relative deviations from parental means) for the major volatiles in trispecies hybrids, as well as in F_1 hybrid *nisy*.

According to the needle volatile composition the hybrids *nisyde* were more similar to the female parent, F_1 hybrid *nisy*, than to the male parent, *P. densiflora* (Figure 6). Based on the analysis of nineteen needle and shoot internal and external morphological traits

Table 2. – Heterosis for the major volatiles in hybrids *nisy* × *de*, *nisy* × *th* and *nisy*, on the basis of parental means.

Compound	heterosis (%)		
	<i>nisy</i> × <i>de</i>	<i>nisy</i> × <i>th</i>	<i>nisy</i>
α -pinene	36.3	11.0	-14.2
camphene	21.8	-19.4	166.7
β -pinene	-25.9	6.7	-22.4
myrcene	-28.8	-7.0	48.1
Δ -3-carene	-92.5	-100.0	35.1
β -phellandrene + limonene	-50.8	28.1	-27.7
α -terpinolene	36.7	-7.3	52.9
bornyl acetate	51.0	14.6	684.6
β -caryophyllene	-14.3	-4.3	25.9
germacrene D	-6.1	-16.4	21.9
<i>endo</i> -1-bourbonanol	9.1	37.3	-35.3

(BORZAN and IDŽOJTIĆ, 2002) the samples of trispecies hybrids *nisyde* could be fully discriminated from the male parent. Discrimination between *nisyde* and female parent *nisy* was not significant. Samples of the trispecies hybrid could be misclassified as samples of hybrid *nisy* in 12% cases.

In view of the needle volatile composition trispecies hybrids *nisyth* were somewhat more similar to the male parent, *P. thunbergiana* (Figure 6). By the analysis of morphological and anatomical needle and shoot traits (BORZAN and IDŽOJTIĆ, 2002) it was possible to fully differentiate the hybrids *nisyth* from the male parent *P. thunbergiana*. Misclassification of the trispecies hybrid samples as samples of the female parent *nisy* was possible in 15% of cases.

The intermediary quality of the hybrids *nisyde* and *nisyth* for most components in relation to their parental species, as well as the previous morphometric analysis, confirms their hybrid character.

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