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MORPHOMETRICAL ANALYSIS AND NEEDLE VOLATILES COMPOSITION OF SOME HARD PINE SPECIES AND THEIR HYBRIDS

MORFOMETRIJSKA ANALIZA I SASTAV ETERIČNIH ULJA IGLICA NEKIH VRSTA BOROVA I NJIHOVIH HIBRIDA

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The paper deals with nineteen morphological and anatomical traits of needles and shoots of three pine species (*Pinus nigra* J. F. Arnold, *P. densiflora* Siebold et Zucc. and *P. thunbergiana* Franco) and their four hybrids (F_1 hybrids *P. nigra* × *P. densiflora*; reciprocal hybrids *P. densiflora* × *P. nigra;* F_1 hybrids *P. nigra* × *P. thunbergiana*, and reciprocal hybrids *P. thunbergiana* × *P. nigra*). The analysed traits are as follows: needle length, fascicle sheath length, one-year shoot tracheid length and width, the number of ventral and dorsal stomatal rows, the number of stomata along one row, the number of serrations along one needle margin; needle cross-section area, needle cross-section height and diameter; stellar region cross-section area; stellar region cross-section height; stellar region cross-section diameter; the largest number of hypodermal cell layers on the needle cross-section; the number of medial resin canals on needle cross-section; the largest and the smallest number of sheath cells surrounding a single resin canal.

The possibility to differentiate the hybrids from the parent species was investigated. Based on the analysed traits, with a probability range of between 95% and 100%, it was possible to discriminate F_1 hybrids *P. nigra* × *P. densiflora, P. densiflora* × *P. nigra*, and *P. thunbergiana* × *P. nigra* from the parent species. Hybrids *P. nigra* × *P. thunbergiana* differ significantly from the male parent, the Japanese black pine, but they do not differ from the female parent, the European black pine. The features, by which it is possible to identify the hybrids, were separated by a discriminant analysis.

Chemical analytical methods (gas chromatography and gas chromatography/mass

spectrometry) were used to determine, in terms of quality and quantity, the composition of needle volatile of the mentioned species and hybrids. Cluster analysis was used to determine to what degree the individual species and hybrids resemble in terms of the composition of the needle volatile. The Japanese black pine and its hybrids (*P. nigra* × *P. thunbergiana* and *P. thunbergiana* × *P. nigra*) differ greatly from other species and hybrids. The second unit consists of two groups. The first is composed of the European black pine and the hybrids *P. nigra* × *P. densiflora*. The second group consists of the Japanese red pine and the hybrids *P. densiflora* × *P. nigra*.

The proportion of α-pinene is the largest of all components in the needle volatile of all analysed species and hybrids (between 25.8% in *P. densiflora* and 42.7% in *P. nigra*), except for the Japanese black pine in which the largest proportion is that of β-pinene (34.1%).

The component which is specific of the European black pine is germacrene D, the one specific of the Japanese red pine is thunbergol, while β-pinene is specific of the Japanese black pine.

In F_1 hybrids the proportion of the individual components is larger, smaller or mostly intermediary between the respective proportions of the parent species. The following are the components whose proportions in all analysed F_1 hybrids are intermediary between the respective proportions of the parent species: α-pinene, β-bourbonene, β-caryophyllene, germacrene D and α-muurolene. These components could be used for the verification of F_1 hybrid plants.

Key words: *Pinus nigra* J. F. Arnold, *P. densiflora* Siebold et Zucc., *P. thunbergiana* Franco, interspecific hybrids, needle morphology, needle anatomy, shoot tracheids, discriminant analysis, volatiles, GC, GC/MS, terpenes, cluster analysis

INTRODUCTION
UVOD

Four pine species, the European black pine (*Pinus nigra* J. F. Arnold), Scots pine (*P. sylvestris* L.), the Japanese red pine (*P. densiflora* Siebold et Zucc.) and the Japanese black pine (*P. thunbergiana* Franco), were used at the Department of Forest Genetics and Dendrology of the Faculty of Forestry, The University of Zagreb, in the period from 1958 until 1991, for the production of the hybrids of F_1 generation, F_2 generation, back-cross hybrids and trispecies hybrids. The research was financed through the collaboration with the U.S. Department of Agriculture. The research results were published in many articles and annual reports as well as in the final reports of the following four projects:

1. The influence of irradiation of pollen on the physiology of growth, 1967-1972;
2. The effect of micro-environment on species incompatibility in hard pines, 1974-1977;
3. The factors of incompatibility between the European black pine and Scots pine and the possibilities of mass production of their hybrids, 1980-1985;
4. Improvement of forest trees, 1986-1991.

The production and evaluation of these plants is a long-term process requiring controlled

hybridisation on trees, a two-year development of cones, sowing of seeds with nursery growing of plants, and establishing of test plots. A large number of the produced hybrid plants were planted on fourteen test plots in the areas of Đurđevački peski and the Lisičine Arboretum.

The research continued within the project "The Breeding of Conifers", which was sponsored by the Ministry of Science and Technology of the Republic of Croatia and conducted by Professor Želimir Borzan. The research was carried out as two projects, both financed by the Ministry of Science and Technology and the Croatian Forests Enterprise: "Hybrids of four pine species and their determination" and "Variability research in various families of interspecific hard pine hybrids".

The growth and development of the hybrid plants in relation to both control plants of pure species and other hybrid combinations have been continually monitored since the establishment of the test plots. Different morphometrical investigations have also been conducted.

This research is a contribution to the evaluation of the hybrid plants produced under control, in terms of the resemblances with the original species displayed by the individual hybrid combinations. Analyses of the needle volatiles of these species and hybrids were also done.

F_1 HYBRIDS *PINUS NIGRA* × *P. DENSIFLORA* (= *NIDE*) AND RECIPROCAL HYBRIDS *P. DENSIFLORA* × *P. NIGRA* (= *DENI*)

F_1 HIBRIDI *PINUS NIGRA* × *P. DENSIFLORA* (= *NIDE*) I RECIPROČNI HIBRIDI *P. DENSIFLORA* × *P. NIGRA* (= *DENI*)

The hybrid *P. nigra* × *P. densiflora* was first produced by Blakeslee in 1914 (Johnson 1939). This hybrid was later produced and described by Wright & Gabriel (1958), Wright (1962), Wright *et al.* (1970), Vidaković (1963, 1966), Vidaković *et al.* (1973). F_1 hybrids between the black and the Japanese red pine often blossom at the age of two or three years. Related to their parents, they are intermediary in terms of length and width of needles, the arrangement of stomata, the location of resin canals and the shape and colour of buds. As to other investigated anatomical traits (height of needle cross-section, number of hypodermal layers and number of sheath cells above the phloem) the hybrids were closer to one of the parents (Vidaković 1966). While young, they grew faster than the black pine and slower than the Japanese red pine (Vidaković 1974). They tolerated well transplantation and were resistant to *Scirrhia acicola*, syn. *Dothistroma pini* (Vidaković *et al.* 1973). When growing one next to the other, these two species often breed spontaneously (Wrigth *et al.* 1970).

When young the hybrids *P. densiflora* × *P. nigra* grew slower than the reciprocal hybrids, and are also more difficult to produce (Vidaković 1974).

The morphometrical needle analysis of these hybrids was conducted by Idžojtić (1996, 1997). The analysis of growth on test plots of twelve-year old plants was done by Borzan *et al.* (1995) and Idžojtić (1996). The respective survival percentages of the hybrids *nide, deni*, the black pine and the Japanese red pine were 67%, 57%, 24% and 59%. The largest average

diameter was 15.4 cm (*nide)*; the respective average diameters of the Japanese red pine, the hybrid *deni* and the black pine were 13.4 cm, 12.1 cm and 9.3 cm.

F_1 HYBRIDS *PINUS NIGRA* × *P. THUNBERGIANA* (= *NITH*) AND RECIPROCAL HYBRIDS *P. THUNBERGIANA* × *P. NIGRA* (= *THNI*)

F_1 HIBRIDI *PINUS NIGRA* × *P. THUNBERGIANA* (= *NITH*) I RECIPROČNI HIBRIDI *P. THUNBERGIANA* × *P. NIGRA* (= *THNI*)

Successful breeding of the European and the Japanese black pine was reported by Wright & Gabriel (1958). The hybrid differed from its female parent, the European black pine, in needles and in its faster growth under nursery conditions.

Reciprocal breeding between the Japanese black pine and the European black pine was also reported by Wright & Gabriel (1958) and Wright (1962). Young plants in a nursery could be discriminated easily from both parents, and they had a small heterotic effect.

It took many years to produce different breeding combinations of two-needle pines at the Department of Forest Genetics and Dendrology of the Faculty of Forestry, University of Zagreb. The results include hybrids of the European and the Japanese black pine, and reciprocal hybrids. Borzan *et al.* (1995) and Idžojtić (1996) analysed the growth of fifteen-year old plants on test plots. The survival of the *nith* hybrids was 100%, while the respective survival percentages of *thni*, the European black pine and the Japanese black pine were 85%, 60% and 50%. The average diameters of the hybrids and the Japanese black pine were nearly the same, or considerably larger, than the diameter of the European black pine (*nith* 11.8 cm; *thni* 11.9 cm; *th* 11.7 cm; *ni* 7.8 cm).

ESSENTIAL OILS
ETERIČNA ULJA

Essential oils are volatile and odorous liquids, hydrocarbon mixtures of different composition. Contained by organs of many plants, they synthesize primarily in plastids. Transparent and colourless, essential oils darken when exposed to air; they dissolve in organic solvents, plant and animal oils and fats, and they do not mix with water. They can be of different composition. More than five hundred compounds can be separated from them (Kramer & Kozlowski 1960). Characteristic constituents of essential oils are terpenes.

More than one thousand of known different essential oils are obtained by distillation, pressing or extraction.

Balms are semi-liquid, while resins are dense or hard mixtures of essential oils (distillable) and resin acids (non-distillable), and of other substances (Denffer & Ziegler, Dell & McComb 1979).

For chemical processing, the only significant essential oils are those obtained from conifers, mainly from their needles. Pine needles contain between 0.4% and 0.5% essential oils, which are obtained by water vapour distillation. Essential oils containing esthers are particularly sweet-smelling and are used in the production of soap, perfumes, disinfectants,

medicine and food industry.

Unlike fats, essential oils do not serve as food supply of plants, but are a secondary product of metabolism with undetermined metabolical role. Since many essential oils are volatile, they are continuously lost from trees through evaporation.

Conifers (*Coniferae*) contain essential oils and resins in all parts of the plant. The *Pinales* order with six hundred species is today the most significant group of gymnosperms. A large number of these species are important for the production of timber. Another economically important role is the production of resins and essential oils. Commercially significant essential oils are obtained from the species of the *Cupresaceae* family which, unlike the

TERPENES
TERPENI

Terpenes are widely spread in nature, above all in plants as components of resins and essential oils. Many terpenes are hydrocarbons, though some of them - alcohols, aldehydes and ketones - also contain oxygen. Their basic unit is the unsaturated hydrocarbon isoprene: CH_2-$C(CH_3)$-CH-CH_2. The common formula of terpene hydrocarbons is $(C_5H_8)_n$. Table 1 shows the division of terpenes per number of isoprene units. By some authors, only hydrocarbons are considered terpenes. In terms of all components, these authors name them terpenoids; however, the term terpene is also generally used with all components (Norin 1964).

Table 1. Compound-classes of terpenes
Tablica 1. Podjela terpena

Compound-class *Vrsta terpena*	No. of Isoprene Units *Broj jedinica izoprena*	Example *Primjer*
Monoterpene	2	pinene, camphor, menthol
Sesquiterpene	3	nerolidol, farnesol
Diterpene	4	vitamin A_1
Triterpene	6	squalene
Tetraterpene	8	caroten

The terpenes in conifers are component parts of the following: the essential oils in the needles (Zavarin 1970, von Rudloff 1975); the resins in the bark (Squillace 1977b, Meier & Goggans 1978) and the wood (Bannister *et al.* 1962; Franklin 1976; Green *et al.* 1974, 1975; Strauss & Critchfield 1982). Each of the sources is an independent system, which means that the resin canals of different plant organs are not interconnected (von Rudloff 1975). The composition of terpenes from needles or bark is in most cases similar (Squillace 1977b,

Schiller & Grunwald 1987a).

Since 1950, chemical methods have been used in taxonomy. Mirov (1961) wrote that terpenes can be useful in differentiating many pine species. Although each species does not have a specific terpene composition, the knowledge about this composition may contribute to the understanding of geographic distribution and evolutionary history of pines. The same author wrote that the composition of terpenes and the taxonomy of the species of genus *Pinus* often coincide.

Among the authors who wrote about the analyses of terpenes and the use of gas chromatography in taxonomic research on pines were Zavarin (1968) and Turner & Flake (1974). Research on plant resins was at first motivated by commercial significance of these resins in some species such as *Pinus elliottii* (Roberts *et al.* 1982). The analysis of terpene composition as research technique became possible with the development of gas chromatography which exactly determines the composition of microgram sample quantities (Simpson 1970, Squillace 1976). The following are some of the written studies important for understanding the analysis of terpene composition: Lever & Burley (1974), von Rudloff (1975), Squillace (1976), Seal *et al.* (1977), Rudin (1979), Burley & Lockhart (1985), Birks & Kanowski (1988, 1993).

Of all plants, conifers produce the largest quantities of terpene. The biosynthesis of terpenes was investigated by Zavarin (1970), Gleizes *et al.* (1980), Bernard - Dagan (1988), Vögeli & Chappell (1990), Lewinsohn *et al.* (1991), Salin *et al.* (1995) and Socaciu *et al.* (1995). Research into the terpene metabolism was conducted by McGarvey & Croteau (1995), Chappell (1995) and others.

Terpene composition analysis is used in chemical taxonomy and in the research on geographic variability. The following are examples of such research on different pine species: *Pinus nigra* - Thorin & Nommik (1974), Gerber *et al.* (1995), Chalchat & Gorunović (1995a, b); *P. pinaster* - Baradat *et al.* (1978); *P. halepensis* Schiller & Grunwald (1987b) and Baradat *et al.* (1995); *P. brutia* - Schiller & Grunwald (1987a) and Schiller & Genzi (1993); *P. mugo* - Prinz (1990).

Of American pine species, the composition of essential oils and resins and the variability of this composition were investigated for the following species: *P. elliottii* (Gansel & Squillace 1976 and Squillace *et al.* 1980a); *P. taeda* (Rockwood 1973 and McRae & Thor 1982); *P. ponderosa* (Smith 1964, 1977, Zavarin & 1970, Adams & Edmunds 1989 and von Rudloff & Lapp 1991); *P. cembroides* (Zavarin & Snajberk 1985); *P. monophylla* (Smith & Preister 1988); *P. contorta* (Forrest 1977, 1987, von Rudloff & Nyland 1979, von Rudloff *et al.* 1985 and von Rudloff & Lapp 1987), *P. albicaulis* (Zavarin *et al.* 1991); *P. radiata* (Cool & Zavarin 1992); *P. monticola* (Townsend *et al.* 1972 and Zavarin *et al.* 1990); *P. strobus* and *P. monticola* (Hunt *et al.* 1990), and for the Central American pine species (Green *et al.* 1974, 1975, Burley & Green 1977, 1979 and Lockhart 1990a, b).

The variability of terpene composition of common pine (*P. sylvestris*), considering its wide distribution and significance, has been thoroughly investigated. Research on this was carried out by the following: Tobolski & Hanover (1971); Juvonen (1970a, b); Juvonen & Hiltunen (1972); Thorin & Nommik (1974), Hiltunen *et al.* (1975a), Hiltunen (1976); Forrest

(1980); Chalchat *et al.* (1985); Yazdani *et al.* (1985); Muona *et al.* (1986); Yazdani & Nilsson (1986); Raitio (1990); Nerg *et al.* (1994); Orav *et al.* (1996) and others.

In order to establish the possibility of using terpene composition in different types of genetic research, it was necessary to investigate the degree of genetic control, i.e. the degree of inheritance of terpene composition. Such research was done for the following species: *P. sylvestris* (Hiltunen 1975, 1976, Hiltunen *et al.* 1975b, Yazdani *et al.* 1982, Baradat & Yazdani 1988, Pohjola *et al.* 1989 and Yazdani & Leberton 1991); *P. monticola* (Hanover 1966a, b); *P. contorta* (White 1984 and White & Nilsson 1984); Hybrids *P. attenuata* × *P. radiata* (Strauss & Critchfield 1982); *P. elliottii* (Squillace 1971, 1977a, b and Squillace & Fisher 1966); *P. virginiana* (Meier & Goggans 1978); *P. taeda* (Squillace *at al.* 1980b and Squillace & 1986) and *P. banksina* (Lapp & von Rudloff 1982).

The relation between the terpene composition and the resistance to fungi was investigated by Peterson & Read (1971), de Groot (1972), Risbeth (1972), Schuck (1982), Michelozzi *et al.* (1990), Himejima *et al.* (1992), and others. The link between terpene composition and resistance to insects was also investigated (Anderson & Fisher 1960, Hanover 1975, Annila & Hiltunen 1977, Wilkinson 1980, Alfaro *et al.* 1980, 1981, Harris *et al.* 1983, Reed *et al.* 1986, Brooks *et al.* 1987a, Delorme & Lieutier 1990).

Besides pines, terpene composition of other conifers has also been investigated. Such research with firs was conducted by Smedman *et al.* (1969), Lee *et al.* (1974), von Rudloff & Grant (1982), Paule *et al.* (1988), Fady *et al.* (1992), Lang (1992, 1994) and Fady (1995). The composition of the terpene from the resin of the shoots of the European and the Japanese larch was investigated by Lang (1989). The research on the composition of the monoterpenes in the bark resin of Norway spruce was conducted by Esteban *et al.* (1976), while the composition of the needle volatile terpene of the same species was investigated by Schönwitz *et al.* (1990) and Orav *et al.* (1996). Terpene composition in the needle essential oils of the Douglas-fir was analysed by von Rudloff & Rehfeldt (1980). Geographic variability of the composition of monoterpenes in the leaves of Californian redwood was investigated by Hall & Langenheim (1987). The composition of essential oils in the leaves of giant arborvitae was analysed by Rudloff *et al.* (1988). The composition of essential oils and resin of some cypress species was investigated by Zavarin *et al.* (1971), Senter *et al.* (1975) and Schiller (1990).

Besides the identification of hybrids through morphological and anatomical traits, it is possible to use the analysis of terpene composition. Gallis & Panetsos (1997) investigated the possibility of differentiating the species *Pinus brutia* and *P. halepensis*, and their F_1, F_2 and the back-cross hybrids governed by the composition of terpene in the bark resin. There was no quality difference, but the differences in terms of quantity in several significant terpenes can be used in differentiating the species and their hybrids.

The concentration of monoterpenes in the air of coniferous forests was investigated by Evans *et al.* (1982), Yokouchi *et al.* (1983), Isidorov *et al.* (1985), Jutner (1986) and Petersson (1988).

MATERIAL
MATERIJAL

The samples for analysis were one-year old, fully developed shoots with needles, collected in late October 1996. The pine trees from which samples were taken grew in Đurđevački peski (four plots) and in the Arboretum Lisičine (five plots), while the parent trees grew in the area of the Faculty of Forestry in Zagreb. Two one-year old shoots were taken from each tree.

The samples were taken from three pine species (*P. nigra*, *P. densiflora* and *P. thunbergiana*) and four combinations of their hybrids (*P. nigra* × *P. densiflora*, *P. densiflora* × *P. nigra*, *P. nigra* × *P. thunbergiana* and *P. thunbergiana* × *P. nigra*). For each group (species and hybrid combinations respectively), the samples were taken from the largest possible number of different trees. Since this possible number of trees differed, the size of the samples differed as well. For the analysis of the European black pine, the shoots were taken from 41 trees; the shoots from forty trees were taken for the analysis of the Japanese red pine, and the shoots from nine trees for the Japanese black pine. The analysis of F_1 hybrid *nide* required samples from 29 trees; the analysis of *deni* required the samples from 10 trees, the *nith* analysis used samples from 15 trees, while the *thni* analysis had samples from 5 trees.

One-year old shoots and needles were used for the morphological and anatomical analysis. The shoots were stored in a biological chamber at 4 °C.

Essential oils were obtained from fresh needles of each of the species and hybrids by five-hour long water vapour distillation. For each species and hybrid, between 10 g and 25 g of cut up needles were distilled with the Karlsruher device (Stahl 1953). The essential oils were stored in 1.0 ml n-pentene and used in further analyses.

THE ANALYZED MORPHOLOGICAL AND ANATOMICAL TRAITS
ANALIZIRANA MORFOLOŠKA I ANATOMSKA OBILJEŽJA

To distinguish hybrids from the parent species, it is necessary to use a combination of several diagnostic characteristic. By means of analysis, the traits, by which the given groups are best distinguished, are separated from the largest possible number of traits. Kriebel (1962) wrote that every hybrid has a different combination of traits by which it is best discriminated, i.e. that once a combination of traits of one interspecific hybrid has been established, it cannot be applied to the hybrids of other pine species.

This paper deals with the following nineteen traits of needles and shoots:

1. *LI* = needle length, in cm, with an accuracy of 0.1 cm. Needles were photocopied, the photocopies were scanned and the needle length was measured by computer, using the Optimas 5.2 program package.
2. *LR* = fascicle sheath length, in cm, with an accuracy of 0.1 cm. Fascicle sheath length was measured in the same way as needle length.
3. *LT* = the wood of the shoot was macerated by cooking for 1-2 minutes in a

10% HNO_3 (according to GERLACH, 1969). The tracheids were then separated and permanent slides were mounted. Tracheid length was measured by Zeiss Axioscope connected to a computer with a video camera, in the *Optimas 5.2* program package.

4. *DT* = one-year shoot tracheid width, in μm, with an accuracy of 0.1 μm. The tracheid width was measured in the middle of the tracheid; the measuring was done in the same way as for the tracheid length.
5. *NPPU* = the number of ventral stomatal rows in the middle of the needle length.
6. *NPPV* = the number of dorsal stomatal rows in the middle of the needle length. The stomatal rows were counted under a binocular magnifying glass with a magnification of 64x.
7. *NP/cm* = the number of stomata along one stomatal row on the inner side of the needle, on a 1 cm-long segment in the centre of the needle (0.5 cm on both sides from the centre of the needle). The stomata were counted under a binocular magnifying glass with a magnification of 64×. An attempt was made to choose the central row. However, if the central stomatal row was interrupted, the closest uninterrupted stomatal row was chosen for the purpose of measuring.

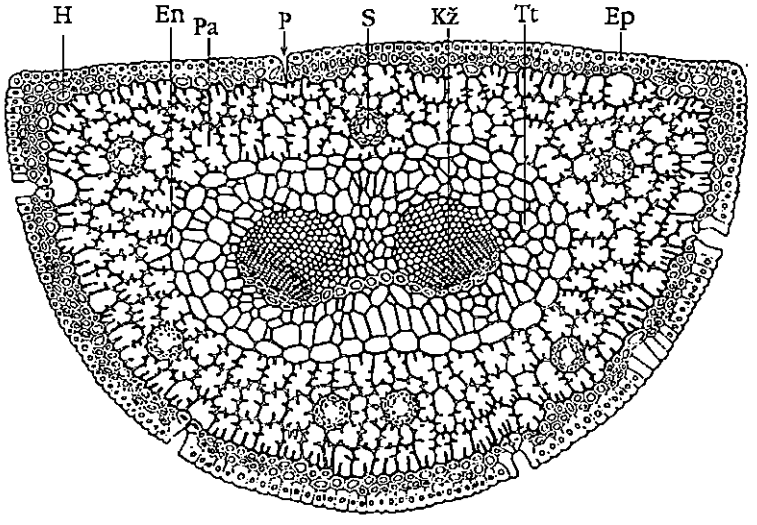

Figure 1. The European black pine needle cross-section. H = hypodermis, En = endodermis, Pa = assimilation parenchyma, P = stoma, S = resin canal, Kž = collateral vessel, Tt = transfusion tissue, Ep = epidermis (according to Denffer & Ziegler 1982)

Slika 1. Poprečni presjek iglice europskoga crnoga bora. H = hipoderma, En = endoderma, Pa = asimilacijski parenhim, P = puč, S = smolenica, Kž = kolateralna žila, Tt = transfuzijsko tkivo, Ep = epiderma (prema Denffer & Ziegler 1982)

8. *NZ/cm* = the number of serrations along one needle margin on a 1 cm-long segment in the centre of the needle (0.5 cm on both sides from the centre of the needle). The serrations were counted under a binocular magnifying glass with a magnification of 64x.
9. *PPP* = needle cross-section area, accuracy 1 μm^2. The traits 9-14 were measured on the cross-section, taken from the middle of the needle length. Permanent cross-section slides were mounted (according to GERLACH, 1969). The needle cross-section and the stellar region cross-section (traits 9-4) were measured by Zeiss Axioscope, connected to a computer with a video camera in the *Optimas 5.2* program package.
10. *HPP* = needle cross-section height, accuracy 1 μm.
11. *DPP* = needle cross-section diameter, accuracy 1 μm.
12. *PCC* = stellar region cross-section area, accuracy 1 μm^2.
13. *HCC* = stellar region cross-section height, accuracy 1 μm.
14. *DCC* = stellar region cross-section diameter, accuracy 1 μm.
15. *NHmax* = the largest number of hypodermal cell layers on the cross-section in the middle of the needle. Hypodermal cell layers were counted on the needle cross-section slides. As the number of these layers varies inside a cross-section, the largest number of layers occurring on one cross-section was recorded.
16. *NSKM* = the number of medial resin canals on the cross-section in the middle of the needle. Resin canals were counted on the needle cross-section slides.
17. *NSKH* = the number of external resin canals on the cross-section in the middle of the needle. The external and medial resin canals were counted on the same needle cross-section slides, that is both traits were recorded for each slide.
18. *NSmax* = the largest number of sheath cells surrounding a single resin canal on the cross-section in the middle of the needle. The cells were counted on the needle cross-section slides. On each cross-section, several resin canals are seen. The size of these canals as well as the number of the surrounding cells vary. On each cross-section the largest and the smallest number of sheath cells surrounding a single canal were recorded (*NSmax* and *NSmin*).
19. *NSmin* = the smallest number of sheath cells surrounding a single resin canal on the cross-section in the middle of the needle.

DISCRIMINANT ANALYSIS
DISKRIMINACIJSKA ANALIZA

The discriminant analysis is one of the statistical analyses which include several variables. Computer has simplified their application. The discriminant analysis is firstly used for finding the variables, by which the previously defined groups may be best discriminated and, secondly, for the classification of new samples into these groups. The result of the analysis are discrimination and classification functions. Though almost at the same time, the discrimination function was independently developed by Mahalanobis (1930), Hotelling (1931) and Fisher (1936).

The theory of discriminant analysis is very complex and comprehensive (Rao 1952, Snedecor & Cochran 1971, Sneath & Sokal 1973, Falkenhagen & Nash 1978, Sokal & Rohlf 1981, Mardia *et. al.* 1982, Kachigan 1991) and will not be explained in this paper.

The basic postulate is that, if the arithmetic means of the variables of different groups differ significantly, a given variable can than be used for the discrimination of these groups, i.e. for the evaluation of the classification of a new sample into one of these groups.

The first example of using a discriminant function with forest trees was the division of two forms of black locust (Hopp 1941).

Clifford & Binet (1954) further developed the discriminant theory for the purpose of classification of the members of the hybrid cluster between two eucalyptus species.

A stepwise discrimination analysis was used for the research into the morphology of pine hybrids (Mergen & Furnival 1960). Besides the description of different morphological characteristics of hybrids and their comparison with the same characteristics of the parent species, Mergen and Furnival wanted to establish which respective characteristics enabled the best discrimination between the hybrids and the parent species. The discriminant analysis was applied in order to find out by which characteristics are the species *P. thunbergiana* and *P. densiflora* distinguished in the best way, then, which are the characteristics that make their F_1 hybrids better than their parents' average, and by which characteristics are these hybrids intermediary. The analysis icluded several traits of outer appearance of the trees, the needles, the top buds and the timber. The traits by which the Japanese black pine is distinguished from the Japanese red pine were the colour of buds, the appearance of bud scales and the number of the serrations along the needle margin. In terms of height and diameter, the hybrids were better than the average of both parents, while the intermediation related to the parent species was best illustrated by the colour of buds.

A similar analysis was applied by Mergen *et al.* (1966) in the differentiation of the saplings and young plants of F_1 hybrids of two eucalyptus species (*Eucalyptus cinerea* × *E. maculosa*), both between the parent species and between these two species.

For distinguishing the provenances *P. taeda* and *P. echinata* as well as these two species from one another, Wells *et al.* (1977) included six needle traits into the discriminant analysis: needle length, needle width, the number of serrations, the number of stomatal rows, the number of stomata along one stomatal row, and the number of resin canals. The two species are mostly distinguished by needle length, although all other traits considerably contribute to

their differentiation.

For distinguishing nine different hybrids between four three-needle pine species (*P. palustris, P. elliottii* var. *elliottii, P. taeda* and *P. echinata*), Snyder & Hamaker (1978) analysed nine morphological and anatomical traits of the needles of four-year old plants. Using discriminant analysis, different traits of four different breeding combinations were separated. For example, for *P. palustris* × *P. elliottii* var. *elliottii*, fascicle sheath length, needle length and the number of stomatal rows were separated, while the number of stomata per length unit, needle diameter and the percentage of resin canals touching the endoderm were all separated for *P. echinata* × *P. elliottii* var. *elliottii*.

By using different analyses with several variables, including the discriminant analysis, Calmasii *et al.* (1988) separated the following four traits by which the mutual discrimination of fourteen populations from different natural ranges of *P. brutia* would be possible: total number of stomata, the number of stomata per length unit, needle width, and the number of hypoderm cell layers.

Besides distinguishing formerly determined groups, discriminant analysis is also used in the classification of new samples into one of the groups. Governed by characteristic forms of branches, leaves and fruits, Solomon & Kenlan (1982) applied discrimination analysis to classify the interspecific and cross-specific hybrids of three birch species, *Betula alleghaniensis, B. papyrifera* and *B. populifolia.*

The mathematical problem of discrimination analysis in terms of choosing the variables whose arithmetic means significantly differs among the groups, can be taken as the problem of variance analysis. If there is only one variable, the calculation is analogous to the single variance analysis (ANOVA - analysis of variance). However, in order to see which of the variables, and to what degree, contributes to the differentiation of the groups, this research, as is usually the case with any other research, includes several variables. Thus, the procedure is analogous to the variance analysis with several variables (MANOVA - multivariate analysis of variance). Although the calculation with several variables is much more complex, the basic principle remains the same: the search for the variables that differ from one another between the groups, which is evident in the differences between the arithmetic means.

If the research involves several variables, those variables that are significant for the discrimination of the groups are analytically separated, and we say that these variables are included into the "model". In this study, the distinguishing of groups (species and breeding combinations respectively) is governed by nineteen variables, while the model includes a different number, since two separate analyses are done. The proportion of how much each of the variables contributes to group differentiation is expressed mathematically.

A case involving two groups mathematically coincides with regression analysis, while a case with several groups is solved by canononical analysis (Namkoong 1967, Burley & Burrows 1972). It is the result of several discriminant functions and canonical roots respectively. They are independent, successive functions, and their contributions to group differentiation do not coincide. The first function expresses the largest group discrimination. It is followed by the second function, etc. The maximum number of functions equals the number of groups minus one, or a number of variables in the analysis, depending on which

number is smaller. The functions are linear and of the following type:

$$a + b_1 x_1 + b_2 x_2 + ... + b_m x_m$$

a = constant

$b_1 - b_m$ = standardised coefficients

$x_1 - x_m$ = dependent variables

The bigger the standardised coefficient (according to its absolute value), the bigger is the contribution of the relating variable to the group discrimination by the specified discrimination function. However, these coefficients do not tell us which groups differ from one another. This can be established for each discriminant function through the arithmetic means of the functions, for every group in turns. It is tested which discriminant functions significantly contribute to group differentiation, and only these functions are included in the interpretation, while others are ignored.

Another important application of the discriminant analysis is the classification of new samples into the existing groups. With the model completed and the discriminant functions made, the issue is, how well can be estimated to which of the groups the new sample belongs. For the sake of classification, a classification function for each group is made. The required characteristics of the new sample are measured; for the function, these characteristics are variables. The measured variables are classified into each of the classification functions, while the sample itself belongs, with the highest probability, to the group where the classification result is the highest.

The discriminant analysis in this research was applied in order to establish which of the given groups (species and their hybrids respectively) are best distinguished on the basis of the combinations of nineteen analysed characteristics. A possibility of classifying new samples into given groups was also presented.

As an illustration of using this analysis for distinguishing pine species and their hybrids (*P. nigra*, *P. sylvestris*, *P. densiflora*, F_1 *nisy*, F_2 *nisy*, F_1 *nide*, F_1 *deni* and F_1 *desy*), Idžojtić (1996, 1997) analysed three morphological characteristics (needles length, number of serrations, number of stomata). Only these characteristics did not suffice for hybrid determination; it was emphasised that for the separation of the best combination of characteristics it was necessary to investigate a larger number of anatomic and morphological traits, which was done in this research. Borzan & Idžojtić (1996) increased the number of analysed characteristics for hybrids F_1 and F_2 *nisy* from three to five (needle length, number of serrations, number of stomata, tree diameter and tree height). The determination accuracy of hybrids F_1 and F_2 was increased from 53% to 66% and from 88% to 98% respectively. This proved the fact that the increase in the number of analysed characteristics increases the accuracy of determination.

GAS CHROMATOGRAPHY
PLINSKA KROMATOGRAFIJA

Gas chromatography is an instrumental chemical method used in separating and analysing chemical mixtures and for identification of simple and complex chemical compounds. Requiring just a small quantity of samples, it is used in both quality and quantity analysis. This method enables analyses of chemically very similar compounds and of those that cannot be analysed with other chemical methods. Gas chromatography is today frequently applied because of its high precision and a relatively simple use. Thus, it may determine the composition of volatiles and resins (von Rudloff 1975). A detailed illustration of this method and its theoretical base can be found in literature, e.g. Deur - Šiftar (1973), Schomburg (1987), Skoog & Leary (1992) and others.

The following equipment was used for the chromatography in this research: DANI 8610 and DANI 8400 capillary gas chromatographs (DANI, Monza, Italy). Each was equipped with a programmed temperature vaporiser (PTV), a flame ionisation detector (FID), and an LDC/Milton Roy CI - 10B integrator (LDC/Milton Roy, Riviera Beach, Florida). The samples were analysed 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; film thickness 0.13 μm) capillary column (Chrompack International BV, Middelburg, Netherlands). The carrier gas (hydrogen) velocity was 43 cm/s, while the column temperature programming was 40 °C - 300 °C, with an increase of 4 °C/min, and 300 °C isothermally for 10 minutes. 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 × 0.32 mm i.d.; film thickness 0.25 μm) capillary column (J & W Scientific, Folsom, California). The carrier gas (hydrogen) velocity was 53 cm/s, while the column temperature programming was 40 °C held for 5 minutes, and then from 40 °C heated at 2.5 °C/min to 250 °C. PTV temperature was 50 °C during the injection, followed by a very rapid heating to 250 °C. The FID was operated at 260 °C.

The described equipment was located in the Institute for Plant Physiology of the University of Graz, Austria.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY
PLINSKA KROMATOGRAFIJA/SPEKTROMETRIJA MASA

Gas chromatography combined with mass spectrometry is certainly one of the most powerful analytical instrumental methods used today in analytical chemistry. Gas chromatography separates the individual components of a mixture, as described above, while mass spectrometry is used for direct identification of mixture components.

Mass spectrometry enables the analysis of complex organic and biological compounds in terms of both quantity and quality. This spectrometry provides information on compound

structure. Very precise and relatively fast, this method requires a small quantity of samples.

GS/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 analysed on a DB-1 (dimethylpolysiloxane; 50 m × 0.20 mm i.d.; film thickness 0.33 μm; J & W Scientific capillary column. Other chromatographic conditions were as follows: carrier gas (helium) at 1 mL/min.; column temperature programming: 50 °C of initial temperature held for 3 minutes, then from 50 °C heated at a speed of 4 °C/min to 320 °C; the temperature of 320 °C was held for 5 minutes. The equipment was supplied with the Wiley 275 Database and was used in sample analysis. It is situated in the Institute for Plant Physiology in Graz, Austria.

CLUSTER ANALYSIS
CLUSTER ANALIZA

Cluster analysis is a statistical analysis including several different algorithms for the classification of primarily unclassified objects. The basic question is how to organise the data into a coherent structure, that is, into a tree. In such classification, the higher degree of association the fewer are the similarities of class members. Cluster analysis is not a typcal statistical test, but a group of different algorithms which classify the data in clusters. The theoretical explanation of this analysis is complex and can be found in individual statistical handbooks, e.g. Jardine & Sibson (1971), Anderberg (1973), Sneath & Sokal (1973), Clifford & Stephanson (1975), Hartigan (1975), Späth (1975), Chatfield & Collins (1980).

In this research we used the hierarchy method of joining, that is, the tree clustering algorithm. The purpose of this algorithm is to join the objects into clusters using some similarity measures or object dissimilarities. A typical result of this method is a hierarchy tree (dendrogram), that is, the objects joined, in terms of similarity, into larger clusters, so that eventually they are all linked together. On the horizontal hierarchy tree, axis x marks the linkage distance. Accordingly, for each node, the distances at which the given objects are joined into a new cluster can be read on the diagram (the place where a new cluster is formed).

In forming a cluster, the distances, i.e. the differences between objects, can be calculated in different ways. The usual method is the calculation by means of the Euclid distance, the geometrical distance in multidimensional space.

At first, all objects are clusters and the distances between them are defined by the Euclid distance. To join the existing clusters, the methods used are the complete linkage method and the method of the furthest neighbour respectively; in other words, the distance between two clusters is determined by the longest distance of any two objects in these clusters.

Cluster analysis is a routine method for the interpretation of the data on the volatile and resin composition. For this purpose it was used with pines (Schiller & Grunwald 1987a; von Rudloff & Lapp 1987; Zavarin *et al.* 1990; Schiller & Genizi 1993; Hiltunen & Laasko 1995) and with other conifers (Schiller 1990, Chang & Hanover 1991, Lang 1994).

In this research, cluster analysis was used to determine which species and hybrids are

similar as to the composition of terpene in needle volatiles. The program used was *Statistica* 5.0.

RESULTS
REZULTATI

THE RESULTS OF HYBRID IDENTIFICATION AND DISCRIMINATION
REZULTATI IDENTIFIKACIJE I RAZLIKOVANJA HIBRIDA

Descriptive statistics
Deskriptivna statistika

The description of each of the nineteen investigated morphological and anatomical characteristics of needles and shoots has been presented in the tables (2-20) for all groups together. The comparisons were made in two separate units, i.e. in two analyses. In the first, the compared groups were *ni*, *de*, *nide* and *deni*, in the second, groups *ni*, *th*, *nith* and *thni*.

The analysis included F- and t- tests for each investigated trait. F-test determined whether, in terms of the analysed characteristics, there are significant differences between the variances of the individual groups. For the groups whose variances do not differ significantly, a t-test was done to establish whether there were significant differences between their arithmetic means (for nineteen analysed traits).

The values of F- and t-tests are expressed by non-significance probabilities of the differences, i.e., if the value in the table is smaller than 0.05, the difference is significant by a probability that is higher than 95%. The testing was carried out in the program *MS Excel* 97.

Needle length, *LI*
Duljina iglica, *LI*

Analysis 1: *ni*, *de*, *nide*, *deni*. F_1 hybrids *nide* (13.0 cm) averagely have longer needles, while F_1 hybrids *deni* (11.5 cm) have shorter needles than both parent species (*ni* 12.4 cm, *de* 12.0 cm) (Table 2). Significant differences were not established for the arithmetic means of groups *ni-nide* and *de-deni*, while for other group combinations there are significant differences between the variances or between the arithmetic means.

Analysis 2: *ni*, *th*, *nith*, *thni*. The Japanese black pine on the average has slightly longer needles (12.7 cm) than the European black pine (12.4 cm), though the difference is not significant (Table 2). Their F_1 hybrids *nith* (10.2 cm) and *thni* (9.6 cm) have shorter needles than both parent species. As to needle length, both hybrids differ significantly from the parent species, though these two hybrids do not differ from one another significantly.

Table 2. Needle length: means (*LI*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

Tablica 2. Duljina iglica: aritmetičke sredine (LI), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama

Group *Grupa*	*LI* (cm)	*s* (cm)	*CV* (%)	*N*
ni	12.4	3.3	26.2	82
de	12.0	1.9	15.5	80
th	12.7	2.7	21.0	36
nide	13.0	2.4	18.4	58
deni	11.5	2.1	17.8	40
nith	10.2	1.9	19.0	30
thni	9.6	1.5	15.7	30

Fascicle sheath length, *LR*

Duljina rukavca oko iglica, *LR*

Analysis 1: *ni, de, nide, deni.* According to the average values, the parent species have equally long fascicle sheaths (*ni* 1.0 cm; *de* 1.0 cm); F*1* hybrids *nide* have longer fascicle sheaths (1.1 cm) than F_1 hybrids *deni* (0.9 cm) (Table 3). These differences are not significant, except for group *de - deni,* whose differences between the arithmetic means are approaching significant values.

Table 3. Fascicle sheath length: means (*LR*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

Tablica 3. Duljina rukavca oko iglica: aritmetičke sredine (LR), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama

Group *Grupa*	*LR* (cm)	*s* (cm)	*CV* (%)	*N*
ni	1.0	0.2	20.0	82
de	1.0	0.2	17.3	80
th	1.1	0.2	14.6	36
nide	1.1	0.3	22.3	58
deni	0.9	0.1	16.5	40
nith	1.0	0.1	14.7	30
thni	1.0	0.2	20.6	30

Analysis 2: *ni, th, nith, thni.* On the average, the European black pine and F_1 hybrids *nith* and *thni* have equally long fascicle sheath lengths (1.0 cm), though slightly shorter than the Japanese black pine (1.1 cm) (Table 3). The differences between the groups are not significant.

One-year shoot tracheid length, *LT*
Duljina traheida jednogodišnjih izbojaka, *LT*

First analysis: *ni, de, nide, deni.* The Japanese red pine has the biggest average lengths of the tracheids (1.232 mm), while the respective values of the black pine are the smallest (1.055 mm). The tracheids of the F_1 hybrid *nide* (1.065 mm) and *deni* (1.087 mm) are, in terms of their mean lengths, between the parent species, though closer to the black pine (Table 4). There are no significant differences between the groups *de-nide* and *nide-deni.* The groups *ni-de* and *ni-nide* differ significantly according to the variances, while the groups *de-nide* and *de-deni* differ as to the arithmetic means.

Table 4. One-year shoot tracheid length: means (*LT*), standard deviations (*s*), variability coefficients (*CV*) and number of measured tracheids (*N*), per groups

*Tablica 4. Duljina traheida jednogodišnjih izbojaka: aritmetičke sredine (*LT*), standardne devijacije (*s*), koeficijenti varijabilnosti (*CV*) i broj mjerenih traheida (*N*) po grupama*

Group *Grupa*	*LT* (mm)	*s* (mm)	*CV* (%)	*N*
ni	1.055	0.114	10.8	82
de	1.232	0.154	12.5	80
th	1.430	0.153	10.7	36
nide	1.065	0.173	16.3	58
deni	1.087	0.145	13.4	40
nith	1.219	0.220	18.1	30
thni	1.366	0.207	15.2	30

Second analysis: *ni, th, nith, thni.* The Japanese black pine has the longest tracheids (1.430 mm), while the shortest are those of the European black pine (1.055 mm). The average values of the tracheids of F1 hybrid *nith* (1.219 mm) and *thni* (1.366 mm) are intermediary between the parent species, while those of hybrid *thni* are closer to the values of the Japanese black pine (Table 4); statistically, they do not differ from it significantly. The differences as to the tracheid length between other groups are significant either according to the variances or according to the arithmetic means.

One-year shoot tracheid width, *DT*
Širina traheida jednogodišnjih izbojaka, *DT*

First analysis: *ni, de, nide, deni.* In relation to the parent species (*ni* 24.1 μm; *de* 21.9 μm), F_1 hybrids *nide* (21.1 μm) have a lower average value of the tracheid width, while F_1 hybrids *deni* have an even smaller value (20.3 μm) (Table 5). While the difference between the groups *nide-deni* is insignificant, the difference between the groups *de-nide* remains at the border of significance.

Table 5. One-year shoot tracheid width: means (*DT*), standard deviations (*s*), variability coefficients (*CV*) and number of measured tracheids (*N*), per groups

*Tablica 5. Širina traheida jednogodišnjih izbojaka: aritmetičke sredine (*DT*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*) i broj mjerenih traheida (*N*) po grupama*

Group *Grupa*	*DT* (μm)	*s* (μm)	*CV* (%)	*N*
ni	24.1	2.9	12.5	82
de	21.9	2.6	12.0	80
th	26.5	2.6	9.8	36
nide	21.1	2.1	9.7	58
deni	20.3	2.5	12.3	40
nith	26.9	5.3	19.5	30
thni	26.5	3.1	13.8	30

Second analysis: *ni, th, nith, thni.* The average tracheid width of the F_1 hybrid *nith* (26.9 μm) is slightly bigger than with the parent species (*ni* 24.1 μm; *th* 26.5 μm) (Table 5). The average tracheid width of F_1 hybrids *thni* (26.5 μm) equals the respective value of the Japanese black pine and is bigger than the average tracheid width of the European black pine. This is the only difference that is not significant.

The number of ventral stomatal row, *NPPU*
Broj pruga puči s unutrašnje strane iglice, *NPPU*

First analysis: *ni, de, nide, deni.* The *nide* hybrids have an equal *NPPU* as the Japanese red pine (7), while the hybrids *deni* have an equal *NPPU* as the black pine (8) (Table 6). There are no significant differences either between groups *de-nide* and *ni-deni* or between the parent species; groups *ni-nide, de-deni* and *ni-deni* differ significantly as to this trait.

Table 6. Number of ventral stomatal rows, in the middle of the needle length: means (*NPPU*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 6. Broj pruga puči s unutrašnje strane iglice, u sredini duljine iglice: aritmetičke sredine (*NPPU*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*NPPU*	*s*	*CV* (%)	*N*
ni	8	1	15.9	82
de	7	1	16.8	80
th	7	1	14.7	36
nide	7	1	17. 6	58
deni	8	1	15.9	40
nith	7	1	17.4	30
thni	9	1	11.7	30

Second analysis: *ni, th, nith, thni.* Hybrids *nith* have an average *NPPU* (7) same as the Japanese black pine, while the *NPPU* of the hybrids *thni* (9) is somewhat higher than with the parent species (*th* 7, *ni* 8) (Table 6). The mutual differences are significant, except for groups *th - nith.*

The number of dorsal stomatal rows, *NPPV*
Broj pruga puči s vanjske strane iglice, *NPPV*

First analysis: *ni, de, nide, deni.* Related to the parent species, F_1 hybrids *nide* (11) have an intermediary average *NPPV*, while F_1 hybrids *deni* (13) have a higher *NPPV* than both parent species (*ni* 12, *de* 10) (Table 7).

Table 7. Number of dorsal stomatal rows, in the middle of the needle length: means (*NPPV*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 7. Broj pruga puči s vanjske strane iglice, u sredini duljine iglice: aritmetičke sredine (*NPPV*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*NPPV*	*s*	*CV* (%)	*N*
ni	12	2	16.7	82
de	10	2	18.6	80
th	13	2	12.8	36
nide	11	2	19.6	58
deni	13	2	14.6	40

Group *Grupa*	*NPPV*	*s*	*CV* (%)	*N*
nith	12	2	14.8	30
thni	15	2	10.3	30

The difference in the number of dorsal stomatal rows on the outer side of the needle of black pine and the hybrid *deni* is not significant, while the differences between other groups are significant.

Second analysis: *ni*, *th*, *nith*, *thni*. F_1 hybrids *nith* have the average *NPPV* same as the European black pine (12) and a somewhat smaller *NPPV* than the Japanese black pine (13). The reciprocal hybrids *thni* have an average *NPPV* (15) bigger than both parent species (Table 7). When all groups are mutually compared, a significant difference is absent only between groups *ni* - *nith*.

The number of stomata along one row, *NP/cm*

Broj puči duž jedne pruge, *NP/cm*

First analysis: *ni*, *de*, *nide*, *deni*. Same as with the previous analysis, an average *NP/cm* in hybrids *nide* (104) and *deni* (103) is intermediary between the respective values of the parent species (*ni* 101, *de* 119), but is closer to the values of the black pine (Table 8). There is no significant difference between groups *ni* - *deni* and *nide* - *deni*, while other groups significantly differ by this trait.

Table 8. Number of stomata along one stomatal row, on the inner side of the needle, on a 1 cm long segment from the middle of the needle: means (*NP/cm*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 8. Broj puči duž jedne pruge, s unutrašnje strane iglice, na isječku duljine 1 cm iz sredine iglice: aritmetičke sredine (*NP/cm*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (*N*) po grupama*

Group *Grupa*	*NP/cm*	*s*	*CV* (%)	*N*
ni	101	9	9.2	82
de	119	10	8.0	80
th	92	8	9.2	36
nide	104	12	11.4	58
deni	103	9	8.5	40
nith	99	7	6.8	30
thni	109	8	7.0	30

Second analysis: *ni*, *th*, *nith*, *thni*. With hybrids F_1 *nith*, the average *NP/cm* (99) is intermediary between the respective values of the parent species (*ni* 101, *th* 92), and is closer to the European black pine. F_1 hybrids *thni* have an average *NP/cm* (109) bigger than both parent species (Table 8). There is a significant difference between all groups, except for the European black pine related to hybrid *nith*.

The number of serrations along one needle margin, *NZ/cm*
Broj zubaca duž jednoga ruba iglice, *NZ/cm*

First analysis: *ni*, *de*, *nide*, *deni*. With F_1 hybrids *nide* (35) and *deni* (40), the average *NZ/cm* is intermediary as to the parent species (*ni* 32, *de* 54), but is closer to the values of the black pine (Table 9). There are significant differences between the variances of the groups *de - deni* and *nide - deni*, while other groups significantly differ by their arithmetic means.

Table 9. Number of serrations along one needle margin, on a 1 cm long segment from the middle of the needle: means (*NZ/cm*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 9. Broj zubaca duž jednoga ruba iglice, na isječku duljine 1 cm iz sredine iglice: aritmetičke sredine (*NZ/cm*), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*NZ/cm*	*s*	*CV* (%)	*N*
ni	32	7	20.8	82
de	54	8	14.6	80
th	60	8	13.0	36
nide	35	8	22.7	58
deni	40	5	13.8	40
nith	37	9	23.8	30
thni	35	4	10.4	30

Second analysis: *ni*, *th*, *nith*, *thni*. Same as in the previous analysis, the average *NZ/cm* is intermediary as to the parent species (*ni* 32, *th* 60), but is closer to the European black pine (Table 9). All groups significantly differ from one another by this trait. Groups *ni - nith*, *ni-thni*, *th- thni* and *nith - thni* differ from one another by the variances, while groups *ni - th* and *th - nith* differ by the arithmetic means.

Needle cross-section area, *PPP*
Površina poprečnoga presjeka iglice, *PPP*

First analysis: *ni*, *de*, *nide*, *deni*. As to the average cross-section area of the F_1, the hybrids *nide* (0.8180 mm²) and *deni* (0.8142 mm²) are very similar, and these values are intermediary between the values of the parent species (*ni* 1.0031 mm²; *de* 0.5935 mm²) (Table 10). All groups are significantly different, except for F_1 hybrid *nide-deni*.

Table 10. Needle cross-section area: means (*PPP*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

Tablica 10. Površina poprečnoga presjeka iglice: aritmetičke sredine (PPP), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama

Group *Grupa*	*PPP* (mm²)	*s* (mm²)	*CV* (%)	*N*
ni	1.0031	0.1985	19.8	82
de	0.5935	0.0903	15.2	80
th	1.0056	0.2011	20.0	36
nide	0.8180	0.1591	19.4	58
deni	0.8142	0.1371	16.8	40
nith	0.9799	0.1817	18.5	30
thni	1.1550	0.1536	13.3	30

Second analysis: *ni, th, nith, thni.* The European black pine (1.0031 mm²) and the Japanese black pine (1.0056 mm²) have approximately same average values of the cross-section area. F_1 hybrids *nith* have somewhat smaller (0.9799 mm²) average cross-section areas than both parent species (Table 10). F_1 hybrids *thni* differ significantly by their arithmetic means from all other groups, while other groups differ significantly from one another by this trait.

Needle cross-section height, *HPP*

Visina poprečnoga presjeka iglice, *HPP*

First analysis: *ni, de, nide, deni.* F_1 hybrids *nide* (0.898 mm) are, as to the average height of the needle cross-section, intermediary between the parent species (*ni* 0.873 mm; *de* 0.898 mm) (Table 11). There is a statistically significant difference in the variances of groups *ni - de* and *de - nide*; as to the arithmetic means, there are differences between groups *ni - nide*, *ni - deni* and *de - deni.* F_1 hybrids *nide* and *deni* do not differ significantly.

Second analysis: *ni, th, nith, thni.* F_1 hybrids *nith* (0.998 mm) are intermediary, while *thni* (1.082 mm) have a higher average needle cross-section height value than the parent species (*ni* 0.959 mm; *th* 1.058 mm) (Table 11). According to the arithmetic means, groups *ni - th, ni - thni* and *nith - thni* differ significantly. There is no significant difference between groups *ni - nith, th - nith, th - thni* and *th - thni.*

Table 11. Needle cross-section height: means (*HPP*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 11. Visina poprečnoga presjeka iglice: aritmetičke sredine (*HPP*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*HPP* (mm)	*s* (mm)	*CV* (%)	*N*
ni	0.959	0.128	13.3	82
de	0.733	0.081	11.0	80
th	1.058	0.148	14.0	36
nide	0.873	0.110	12.6	58
deni	0.898	0.092	10.3	40
nith	0.998	0.119	11.9	30
thni	1.082	0.111	10.2	30

Needle cross-section diameter, *DPP*
Promjer poprečnoga presjeka iglice, *DPP*

First analysis: *ni*, *de*, *nide*, *deni*. Average needle cross section diameters of F_1 hybrids *nide* (1.313 mm) and *deni* (1.308 mm) are similar, and are intermediary between the parent species (*ni* 1.445 mm; *th* 1.135 mm) (Table 12). All groups differ from one another significantly either as to the variances or as to the arithmetic means, except for F_1 hybrids *nide* and *deni*.

Table 12. Needle cross-section diameter: means (*DPP*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 12. Promjer poprečnoga presjeka iglice: aritmetičke sredine (*DPP*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*DPP* (mm)	*s* (mm)	*CV* (%)	*N*
ni	1.445	0.148	10.3	82
de	1.135	0.088	7.8	80
th	1.379	0.138	10.0	36
nide	1.313	0.130	9.9	58
deni	1.308	0.105	8.0	40
nith	1.414	0.148	10.5	30
thni	1.520	0.136	8.9	30

Second analysis: *ni*, *th*, *nith*, *thni*. The needles of F_1 hybrid *nith* are intermediary as to the average width (1.414 mm), while those of the hybrid *thni* (1.520 mm) are wider than

with both parent species (*ni* 1.445 mm; *th* 1.379 mm) (Table 12). As to the arithmetic means, groups *ni - th*, *ni - thni*, *th - thni* and *nith - thni* differ significantly. There is no significant difference between groups *ni - nith* and *th - thni*.

Stelar region cross-section area, *PCC*
Površina poprečnoga presjeka centralnoga cilindra, *PCC*

First analysis: *ni*, *de*, *nide*, *deni*. Hybrids *nide* (0.2153 mm²) and *deni* (0.2250 mm²) have an average *PCC* that is intermediary between the respective values of the parent species (*ni* 0.2724 mm²; *th* 0.1479 mm²) (Table 13). All groups differ from one another significantly, except for hybrids *nide* and *deni*.

Table 13. Stellar region cross-section area: means (*PCC*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 13. Površina poprečnoga presjeka centralnoga cilindra: aritmetičke sredine (*PCC*), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*PCC* (mm²)	*s* (mm²)	*CV* (%)	*N*
ni	0.2724	0.0577	21.2	82
de	0.1479	0.0335	22.7	80
th	0.2501	0.0589	23.5	36
nide	0.2153	0.0499	23.2	58
deni	0.2250	0.0399	17.7	40
nith	0.2501	0.0443	17.7	30
thni	0.3032	0.0426	14.1	30

Second analysis: *ni*, *th*, *nith*, *thni*. The average *PCC* of F_1 hybrids *nith* (0.2501 mm²) is same as with the Japanese black pine and smaller than with the European black pine (0.2724 mm²). The reciprocal hybrids *thni* (0.3032 mm²) have an average *PCC* that is higher than both parent species (Table 13). As to this trait, hybrids *thni* differ significantly from both parent species, while hybrids *nith* do not differ either from the European or from the Japanese black pine.

Stelar region cross-section height, *HCC*
Visina poprečnoga presjeka centralnoga cilindra, *HCC*

First analysis: *ni*, *de*, *nide*, *deni*. F_1 hybrids *nide* (0.376 mm) and *deni* (0.384 mm) have average *HCC*s that are intermediary between the parent species (*ni* 0.427 mm; *de* 0.319 mm) (Table 14). All groups differ from one another significantly, except for hybrids *nide - deni*.

Table 14. Stellar region cross-section height: means (*HCC*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

Tablica 14. *Visina poprečnoga presjeka centralnoga cilindra: aritmetičke sredine (*HCC*), standardne devijacije (*s*), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (*N*) po grupama*

Group *Grupa*	*HCC* (mm)	*s* (mm)	*CV* (%)	*N*
ni	0.427	0.046	10.7	82
de	0.319	0.042	13.2	80
th	0.452	0.060	13.3	36
nide	0.376	0.040	10.6	58
deni	0.384	0.043	11.1	40
nith	0.424	0.044	10.4	30
thni	0.449	0.024	5.4	30

Second analysis: *ni*, *th*, *nith*, *thni*. F_1 hybrid *nith* (0.424 mm) has a smaller average *HCC* than both parent species (*ni* 0.427 mm; *th* 0.452 mm); it is closer to the values of the European pine. The reciprocal hybrids *thni* (0.449 mm) have an average *HCC* that is intermediary between the parent species, though closer to the Japanese black pine (Table 14). Except for groups *ni* - *nith*, there are significant differences between other groups, either as to the variances (*ni* - *thni*, *th* - *thni*, *nith* - *thni*) or as to the arithmetic means (*ni* - *th*, *th* - *nith*).

Stelar region cross-section diameter, *DCC*

Promjer poprečnoga presjeka centralnoga cilindra, *DCC*

First analysis: *ni*, *de*, *nide*, *deni*. F_1 hybrids *nide* (0.717 mm) and *deni* (0.743 mm) are intermediary, as to this trait, between the respective values of the parent species (*ni* 0.796 mm; *de* 0.591 mm) (Table 15). All groups differ from one another significantly.

Second analysis: *ni*, *th*, *nith*, *thni*. F_1 hybrids *nith* (0.745 mm) have an intermediary average *DCC*, while the reciprocal hybrids *thni* (0.855 mm) have a bigger average *DCC* than the parent species (*ni* 0.796 mm; *th* 0.704 mm) (Table 15). As to this trait, all groups differ from one another significantly, except for the Japanese black pine and hybrid *nith*.

Table 15. Stellar region cross-section diameter: means (*DCC*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 15. Promjer poprečnoga presjeka centralnoga cilindra: aritmetičke sredine (*DCC*), standardne devijacije (*s*), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (*N*) po grupama*

Group *Grupa*	*DCC* (mm)	*s* (mm)	*CV* (%)	*N*
ni	0.796	0.101	12.7	82
de	0.591	0.063	10.6	80
th	0.704	0.089	12.7	36
nide	0.717	0.101	14.1	58
deni	0.743	0.070	9.5	40
nith	0.745	0.086	11.6	30
thni	0.855	0.090	10.5	30

The largest number of hypodermal cell layers on the needle cross-section, *NHmax*
Najveći broj slojeva hipoderme na poprečnom presjeku iglice, *NHmax*

First analysis: *ni*, *de*, *nide*, *deni*. F_1 hybrids *nide* (2.4) and *deni* (2.5) have an intermediary average *NHmax* between the respective values of the parent species (*ni* 3.4; *de* 1.3) (Table 16). All groups differ from one another significantly, except for F_1 *nide* and *deni*.

Table 16. The largest number of hypodermal cell layers on the needle cross-section: means (*NHmax*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 16. Najveći broj slojeva hipoderme na poprečnom presjeku iglice: aritmetičke sredine (*NHmax*), standardne devijacije (*s*), koeficijenti varijabilnosti (*CV*) i broj mjerenih iglica (*N*) po grupama*

Group *Grupa*	*NHmax*	*s*	*CV* (%)	*N*
ni	3.4	0.69	20.6	82
de	1.3	0.47	35.6	80
th	3.1	0.55	18.0	36
nide	2.4	0.50	20.6	58
deni	2.5	0.51	20.3	40
nith	3.1	0.72	23.3	30
thni	3.0	0.45	15.2	30

Second analysis: *ni*, *th*, *nith*, *thni*. F_1 hybrids *nith* have the same average *NHmax* as the Japanese black pine (3.1), and smaller than the European black pine (3.4). The reciprocal hybrids *thni* (3.0) have a smaller average *NHmax* than both parent species (Table 16). Groups *th* - *nith* and *th* - *thni* do not differ significantly, while other groups differ from one another either significantly or as to the variances, or as to the arithmetic means.

The number of medial resin canals on needle cross-section, *NSKM*
Broj medijalno smještenih smolnih kanala na poprečnom presjeku iglice, *NSKM*

The medial resin canals of the Japanese red pine are not normally distributed, but rather according to Poisson's distribution. This is because in these species the resin canals are located near the hypoderm, while only some of them are medial. For this species, the mode and the median has been calculated as the measure of central tendency, while interquartile is the measure of variability (Table 17). An interquartile is the range between the upper and the lower quartile (Q_3 - Q_1). In the same way as the median divides the distribution members in two equal parts, quartiles divide these two parts into equal parts. Down to the lower quartile there are 25% of the distribution members, while up to the upper quartile there are 75% of the distribution members (Serdar 1961, Holman 1969).

Table 17. Number of medial resin canals on the needle cross-section: means (*NSKM*), standard deviations (*s*), variability coefficients (*CV*), mode, median (*M*), interquartile (*range* Q_3 - Q_1) and number of measured needles (*N*), per groups

*Tablica 17. Broj medijalno smještenih smolnih kanala na poprečnom presjeku iglice: aritmetičke sredine (*NSKM*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*), mod, medijana (M), interkvartil (*raspon Q_3 - Q_1*) i broj mjerenih iglica (*N*) po grupama*

Group *Grupa*	*NSKM*	*s*	*CV* (%)	Mode *mod*	*M*	Q_3 - Q_1	*N*
ni	6.0	2.63	45.0				82
de				0	0	0	80
th	4.6	2.11	45.6				36
nide	5.6	2.22	39.8				58
deni	4.1	2.18	53.4				40
nith	5.5	1.72	31.3				30
thni	8.9	2.26	25.3				30

In terms of this trait, Table 17 shows the arithmetic means, the standard deviation, the variability coefficients and the number of measured needles of other species and hybrids.

First analysis: *ni*, *de*, *nide*, *deni*. According to the number of medial resin canals, hybrids *nide* and *deni* bear more resemblance to the black pine. The arithmetic means are *ni* = 6, *nide* = 5.6 and *deni* = 4.1 (Table 17). Groups *ni* - *nide* do not differ significantly, while groups *ni* - *deni* and *nide* - *deni* statistically differ significantly as to the arithmetic means. The Japanese red pine has resin canals located near the hypoderm, so that the mode, median and interquartile are zero.

Second analysis: *ni*, *th*, *nith*, *thni*. Hybrids *nith*, on the average, have an intermediary number of medial resin canals according to the parent species, while hybrids *thni* have a larger number of medial resin canals than both parent species (*ni* = 6.0; *th* = 4.6; *nith* = 5.5; *thni* = 8.9) (Table 17). Groups *ni* - *nith* differ significantly in variances, while other groups

differ significantly in the arithmetic means.

The number of external resin canals on needle cross-section, *NSKH*

Broj uz hipodermu smještenih smolnih kanala na poprečnom presjeku iglice, *NSKH*

It may be supposed that the number of external resin canals in the European black pine, the Japanese black pine and their hybrids *nith* and *thni* are arranged according to Poisson's distribution. In these groups, resin canals are medial, while only some of them are situated near the hypoderm. In these groups, the mode and the median have been calculated as the measure of central tendency, while interquartile is the measure of variability (Table 18). Table 18 shows arithmetic means, standard deviation, variability coefficients and the number of measured needles of other groups.

Table 18. Number of external resin canals on the needle cross-section: means (*NSKH*), standard deviations (*s*), variability coefficients (*CV*), mode, median (*M*), interquartile (*range* Q_3 - Q_1) and number of measured needles (*N*), per groups

*Tablica 18. Broj uz hipodermu smještenih smolnih kanala na poprečnom presjeku iglice: aritmetičke sredine (*NSKH*), standardne devijacije (s), koeficijenti varijabilnosti (*CV*), mod, medijana (*M*), interkvartil (*raspon Q_3 - Q_1*) i broj mjerenih iglica (*N*) po grupama*

Group *Grupa*	*NSKH*	*s*	*CV* (%)	Mode *mod*	*M*	$Q_3 - Q_1$	*N*
ni				0	0	0	82
de	6.5	1.44	22.3				80
th				0	0	0	36
nide	2.1	1.97	93.1				58
deni	2.9	1.49	51.0				40
nith				0	0	0	30
thni				0	0	1	30

First analysis: *ni*, *de*, *nide*, *deni*. Hybrids *nide* and *deni*, according to the number of medial resin canals, are intermediary as to the parent species (the arithmetic means are *de* = 6.5; *nide* = 2.1; *deni* = 2.9; mode and median of *ni* = 0). The variability coefficient of *nide* = 93%, and of *deni* = 51% (Table 18). Groups *de* - *nide* differ significantly in variances, while groups *de* - *deni* and *nide* - *deni* differ as to the arithmetic means.

Second analysis: *ni*, *th*, *nith*, *thni*. The European black pine, the Japanese black pine and their hybrids have medial resin canals, so that the mode and the median of *NSKH* are zero. The interquartile of *ni*, *th* and *nith* is zero, while for *thni* it is one (Table 18). The maximum number of external resin canals on a needle cross section in the European black pine is one, while in other groups this number is three.

The largest number of sheath cells surrounding a single resin canal, on needle cross-section, *NSmax*

Najveći broj ovojnih sklerenhimskih stanica koje okružuju jedan smolni kanal, *NSmax*

First analysis: *ni*, *de*, *nide*, *deni*. An average *NSmax* of the F_1 hybrid *nide* (13.9) and *deni* (14.1) is larger than in both parent species (*ni* 13.4; *sy* 11.6) (Table 19). As to this trait, groups *ni* - *de*, *de* -*nide* and *de* - *deni* differ significantly.

Table 19. The largest number of sheath cells surrounding a single resin canal, on the needle cross-section: means (*NSmax*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 19. Najveći broj sklerenhimskih stanica koje okružuju jedan smolni kanal na poprečnom presjeku iglice: aritmetičke sredine (*NSmax*), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*NSmax*	*s*	*CV* (%)	*N*
ni	13.4	2.17	15.9	82
de	11.6	2.16	18.6	80
th	11.3	1.96	17.3	36
nide	13.9	2.11	15.2	58
deni	14.1	1.70	12.0	40
nith	12.8	2.28	17.8	30
thni	11.7	1.18	10.1	30

Second analysis: *ni*, *th*, *nith*, *thni*. As to this trait, F_1 hybrids *nith* (12.8) and *thni* (11.7) are intermediary between the parent species (*ni* 13.4; *sy* 11.6). The average value of *NSmax* is by *nith* closer to the European black pine, and by *thni* closer to the Japanese black pine (Table 19). There are significant differences in either variances or the arithmetic means among all groups, except for the group *ni* - *nith*.

The smallest number of sheath cells surrounding a single resin canal, on needle cross-section, *NSmin*

Najmanji broj ovojnih sklerenhimskih stanica koje okružuju jedan smolni kanal, *NSmin*

First analysis: *ni*, *de*, *nide*, *deni*. An average *NSmin* of both F_1 hybrids (*nide* 8.5; *deni* 8.8) is larger than in both parent species (*ni* 8.3; *sy* 7.3) (Table 20). As to this trait, the variances of groups *de* – *nide*, *de* - *deni*, and the arithmetic means of groups *ni* - *de* differ significantly.

Second analysis: *ni*, *th*, *nith*, *thni*. The average *NSmin* value of hybrids *nith* (7.9) is intermediary between the parent species (*ni* 8.3; *th*7.8), while in hybrids *thni* (7.0) it is smaller than in both parent species (Table 20). There are significant differences among all

groups, either in the variances (*ni - nith, ni - thni, th - nith, th - thni*), or in the arithmetic means (*ni - th, nith - thni*).

Table 20. The smallest number of sheath cells surrounding a single resin canal, on the needle cross-section: means (*NSmin*), standard deviations (*s*), variability coefficients (*CV*) and number of measured needles (*N*), per groups

*Tablica 20. Najmanji broj sklerenhimskih stanica koje okružuju jedan smolni kanal na poprečnom presjeku iglice: aritmetičke sredine (*NSmin*), standardne devijacije (s), koeficijenti varijabilnosti (CV) i broj mjerenih iglica (N) po grupama*

Group *Grupa*	*NSmin*	*s*	*CV* (%)	*N*
ni	8.3	1.44	16.7	82
de	7.3	1.74	23.8	80
th	7.7	1.43	18.7	36
nide	8.5	1.19	14.0	58
deni	8.8	1.21	13.9	40
nith	7.9	0.88	11.1	30
thni	7.0	0.72	10.3	30

Discriminant analysis
Diskriminacijska analiza

Two separate analyses were done according to how the hybrids and their parent species were grouped. The following were the input parameters for the discriminant analyses:

First analysis:

Grouping variables: *ni*, *de*, *nide* and *deni*;

Independent variables: nineteen traits

Second analysis:

Grouping variables: *ni*, *th*, *nith* and *thni*;

Independent variables: nineteen traits.

Independent variables are used for group discrimination. They are inserted into the model successively, by a *forward stepwise method*. The tolerance for all analyses was 0.01.

First analysis: *ni*, *de*, *nide*, *deni*
Prva analiza: *ni*, *de*, *nide*, *deni*

The analysed issue was the possibility of discriminating the F_1 hybrids *nide* and *deni* according to their parent species, the black pine and the Japanese red pine. Nineteen morphological and anatomic characteristics were included into the analysis.

According to the total data, we can say that there is a significant group discrimination.

Wilks' $\lambda = 0.03$; $F = 31.9$ (degrees of freedom 51 and 715); $p < 0.01$. The model included seventeen variables, except for *HPP* (the height of needle cross-section) and *PCC* (stellar region cross-section area), since they are not significant.

With seventeen variables and four groups, using a canonical analysis, we obtained three discriminant functions. All three functions are significant, i.e. we have three explanations how, based on seventeen variables, we can distinguish four groups (*ni*, *sy*, *nide* and *deni*).

Table 21 shows the variables in order of the contribution to group discrimination of three discriminant functions. Together with the variables, there are corresponding standardised coefficients. The discrimination defined by discriminant function 1 is mostly determined by variable *PPP*, which is followed in turns by *NSKH*, *DPP*, *NZ/cm*, *DCC*, *NHmax*, etc. The discriminant function 2 is mostly determined by variables *PPP*, *DCC*, *NSKM*, *DT*, *HCC*, *NSmax*, etc., while the discriminant function 3 is determined by variables *DCC*, *PPP*, *LR*, *DPP*, *NPPV*, *NSKM*, etc.

Table 21. Order of the variables determining most the discrimination defined by three discriminant functions, and the standardized coefficients of discriminant functions for these variables

Tablica 21. Redoslijed varijabla koje najviše određuju razlikovanje definirano trima diskriminacijskim funkcijama i standardizirani koeficijenti diskriminacijskih funkcija za te varijable

No. *Red. br.*	Discriminant Function 1 *Diskriminacijska funkcija 1*		Discriminant Function 2 *Diskriminacijska funkcija 2*		Discriminant Function 3 *Diskriminacijska funkcija 3*	
	Variable *Varijabla*	Stand. Coeff. *Stand. koef.*	Variable *Varijabla*	Stand. Coeff. *Stand. koef.*	Variable *Varijabla*	Stand. Coeff. *Stand. koef.*
1	*PPP*	- 0.988301	*PPP*	1.836850	*DCC*	- 1.00939
2	*NSKH*	- 0.585851	*DCC*	- 0.833746	*PPP*	0.66816
3	*DPP*	0.557006	*NSKM*	- 0.731004	*LR*	0.55862
4	*NZ/cm*	- 0.441530	*DT*	0.572537	*DPP*	0.54113
5	*DCC*	0.433314	*HCC*	- 0.492681	*NPPV*	- 0.41219
6	*NHmax*	0.413357	*NSmax*	- 0.428435	*NSKM*	0.40622
7	*HCC*	0.313263	*NSmin*	- 0.395780	*HCC*	- 0.37043
8	*LR*	- 0.280353	*NHmax*	0.387855	*NP/cm*	0.27708
9	*LT*	- 0.266299	*LI*	0.307097	*NPPU*	- 0.27578
10	*DT*	0.253828	*NZ/cm*	0.276056	*LI*	0.24078
11	*NSmin*	0.183619	*NPPU*	0.244271	*NZ/cm*	- 0.18117
12	*NPPU*	- 0.179180	*NSKH*	- 0.228395	*NHmax*	- 0.16730
13	*NSKM*	0.152688	*LT*	0.215444	*DT*	0.13307
14	*NPPV*	0.138614	*NPPV*	- 0.200981	*NSmin*	- 0.07626
15	*NSmax*	0.127360	*LR*	- 0.181548	*NSKH*	0.04269
16	*NP/cm*	- 0.072804	*DPP*	- 0.084881	*NSmax*	0.03060
17	*LI*	- 0.023516	*NP/cm*	0.050834	*LT*	- 0.02871
Cumul. Prop. *Kumul. prop.*		0.8493		0.9546		1.00

The last row in Table 21 shows a cumulative proportion of the explained variance of each function. The first function calculates 85% of the explained variance; the second calculated 10%, and the third the remaining 5%.

The means of the canon variables (Table 22) shows between which groups an individual function is discriminant.

Table 22. Means of variables for three discriminant functions, per groups

Tablica 22. Sredine kanonskih varijabla za tri diskriminacijske funkcije po grupama

Group *Grupa*	Discr. Function 1 *Diskr. funkcija 1*	Discr. Function 2 *Diskr. funkcija 2*	Discr. Function 3 *Diskr. funkcija 3*
ni	3.29528	1.07724	- 0.00793
de	- 4.29376	0.55715	0.02970
nide	0.84846	- 1.37805	- 0.93600
deni	0.60192	- 1.32448	- 1.40033

The first discriminant function distinguishes best the pure species, since the means of these groups are the most distant from one another (Table 22). The means of F_1 hybrids *nide* and *deni* are very close to one another and are situated between the means of the parent species, though slightly closer to the black pine. This is also seen in Figure 2, where the individual values of the first function, per group, are shown on the x-coordinate.

The second discriminant function distinguishes best F_1 hybrids *deni* and *nide* (Table 22) of the black pine. This function is graphically presented in Figure 2, coordinate y.

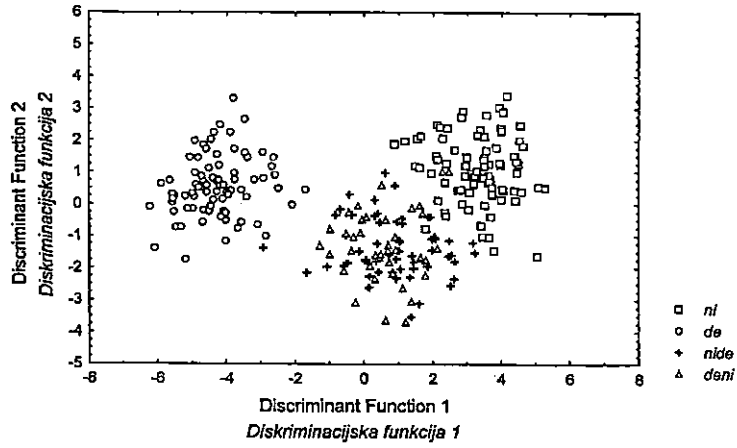

Figure 2. Graph of discriminant functions 1 and 2. Particular values for the first discriminant function are plotted on axis x, and for the second discriminant function on axis y.

Slika 2. Grafički prikaz diskriminacijskih funkcija 1 i 2. Na osi x su pojedinačne vrijednosti za prvu diskriminacijsku funkciju, a na osi y za drugu diskriminacijsku funkciju

The third discriminant function distinguishes best hybrids *nide* and *deni*, though the differences in the means are almost negligible (Table 22). This function includes only 5% of the explained variance.

The coefficients of the classification functions per group are shown in Table 23.

Table 23. Coefficients of classification functions, per groups

Tablica 23. Koeficijenti klasifikacijskih funkcija po grupama

Variable *Varijabla*	*sy*	*de*	*nide*	*deni*
NSKH	- 2.556	0.771	- 1.074	- 1.05
NHmax	14.02	7.77	10.056	10.634
NZ/cm	- 0.011	0.436	0.021	0.098
DT	4.013	3.153	3.276	3.143
NSmax	2.44	2.085	2.809	2.748
LR	- 2.013	9.375	6.41	0.085
DCC	- 40.346	- 74.342	- 39.946	- 14.012
PPP	- 542.47	- 498.992	- 552.14	- 560.207
NSKM	4.331	3.954	5.224	4.717
NSmin	1.686	0.87	1.995	2.072
NPPV	2.028	1.544	1.91	2.372
HCC	595.247	545.693	597.417	615.099
LT	0.024	0.037	0.025	0.026
NPPU	0.998	1.983	0.658	1.224
LI	0.419	0.431	0.223	- 0.002
DPP	545.345	510.785	540.001	528.327
NP/cm	1.341	1.395	1.372	1.309
Constant *Konstanta*	- 440.098	- 406.029	- 419.331	- 417.596

In the classification matrix (Table 24), all measured data are assorted in the classification functions and, according to them, arranged in groups to which they most probably belong (the rows in the table).

Table 24. Classification matrix

Tablica 24. Klasifikacijska matrica

Group *Grupa*	Percent Correct *Točno klasific.* %	*ni*		*de*		*nide*		*deni*		Total *Ukupno*
		No. *kom.*	%	No. *kom.*	%	No. *kom.*	%	No. *kom.*	%	No. *kom.*
ni	98.8	81	98.8	0	0	1	1.2	0	0	82
de	100.0	0	0	80	100	0	0	0	0	80
nide	89.7	0	0	1	1.7	52	89.7	5	8.6	58
deni	82.5	3	7.5	0	0	4	10	33	82.5	40

The classification matrix shows that, based on seventeen analysed characteristics, a safe sample classification of the Japanese red pine and the black pine is possible. There is a possibility of confusion of the black pine samples with hybrids *nide* in 1.2% of cases.

The accuracy of the classification of hybrids *nide* and *deni* is not significant. The samples of hybrid *nide* will be precisely classified in 89.7% of cases, and their misclassification as samples of the Japanese red pine (1.7%) and as reciprocal hybrids *deni* (8.6%) is possible. Based on seventeen analysed characteristics, the samples of hybrid *nide* can be perfectly discriminated from the black pine.

Hybrids *deni* will be accurately assorted with a probability of 82.5%. It is possible to mistake them for black pine (7.5%) and for hybrids *nide* (10%). The samples of hybrids *deni* can be perfectly discriminated from the samples of the Japanese red pine.

The accuracy of hybrid classification is higher if they are separately compared to the parent species. Thus, there is no possibility of mutual replacement of the hybrid samples.

The analysis of F_1 hybrids *nide*, the black pine and the Japanese red pine makes the accuracy of hybrid classification statistically significant (level 0.05), amounting to 96.6% (Table 25). In 1.7% of cases, the samples *nide* can be mistaken for the parent species.

Table 25. Classification matrix
Tablica 25. Klasifikacijska matrica

Group *Grupa*	Percent Correct *Točno klasific.* %	*ni*		*de*		*nide*		Total *Ukupno*
		No. *kom.*	%	No. *kom.*	%	No. *kom.*	%	No. *kom.*
ni	98.8	81	98.8	0	0	1	1.2	82
de	100	0	0	80	100	0	0	80
nide	96.6	1	1.7	1	1.7	56	96.6	58

With the analysis of F_1 hybrids *deni*, the black pine and the Japanese red pine, the limit of statistical significance (level 0.05) of accurate classification of hybrid *deni* is obtained (95%) (Table 25). In 5% of cases, the samples can be mistaken for black pine (Table 26).

Table 26. Classification matrix
Tablica 26. Klasifikacijska matrica

Group *Grupa*	Percent Correct *Točno klasific.* %	*ni*		*de*		*deni*		Total *Ukupno*
		No. *kom.*	%	No. *kom.*	%	No. *kom.*	%	No. *kom.*
ni	100	82	100	0	0	0	0	82
de	100	0	0	80	100	0	0	80
deni	95	2	5	0	0	38	95	40

Second analysis: *ni, th, nith, thni*
Druga analiza: *ni, th, nith, thni*

The analysed issue was the possibility of discriminating the F_1 hybrids *nith* and *thni* according to their parent species, the European black pine and the Japanese black pine. Nineteen morphological and anatomic characteristics were included into the analysis.

According to the total data, we can say that there is a significant group discrimination. Wilks' $\lambda = 0.03$; $F = 20.1$ (degrees of freedom 51 and 468); $p < 0.01$. The model included seventeen variables, except for *HCC* (stellar region cross-section height) and *PCC* (stellar region cross-section area).

Using the canonical analysis, we obtained three discriminant functions. All three functions were significant, i.e. we had three explanations how, based on seventeen variables, we could discriminate four groups (*ni*, *sy*, *nith* and *thni*).

Table 27. Order of the variables determining most the discrimination defined by three discriminant functions, and the standardized coefficients of discriminant functions for these variables

Tablica 27. Redoslijed varijabla koje najviše određuju razlikovanje definirano trima diskriminacijskim funkcijama i standardizirani koeficijenti diskriminacijskih funkcija za te varijable

No. *Red. br.*	Discriminant Function 1 *Diskriminacijska funkcija 1*		Discriminant Function 2 *Diskriminacijska funkcija 2*		Discriminant Function 3 *Diskriminacijska funkcija 3*	
	Variable *Varijabla*	Stand. Coeff. *Stand. koef.*	Variable *Varijabla*	Stand. Coeff. *Stand. koef.*	Variable *Varijabla*	Stand. Coeff. *Stand. koef.*
1	*DCC*	- 1.22007	*DPP*	- 0.857787	*DPP*	2.25252
2	*DPP*	0.90276	*PPP*	0.666934	*PPP*	- 1.69909
3	*NZ/cm*	0.85000	*LT*	0.520485	*DCC*	- 1.22032
4	*HPP*	0.58510	*LI*	- 0.469253	*HPP*	1.06493
5	*NSKM*	- 0.48740	*NPPV*	0.357597	*LI*	- 0.52102
6	*NP/cm*	- 0.44809	*NSmax*	- 0.339467	*NPPU*	- 0.43371
7	*NSmin*	- 0.31970	*NSKM*	0.307173	*LR*	0.41895
8	*LT*	0.31664	*NPPU*	0.281477	*DT*	0.40115
9	*NPPU*	- 0.28122	*NP/cm*	0.276206	*NZ/cm*	- 0.33899
10	*LR*	0.24069	*DCC*	0.261962	*NSKH*	0.29653
11	*LI*	- 0.17715	*NSKH*	0.260501	*NSmax*	0.29006
12	*NPPV*	0.17178	*LR*	0.205430	*NHmax*	- 0.28417
13	*PPP*	- 0.10307	*NHmax*	- 0.201303	*NSKM*	- 0.21410
14	*DT*	0.04062	*NZ/cm*	- 0.198195	*LT*	- 0.15167
15	*NSmax*	- 0.02233	*DT*	0.139214	*NSmin*	- 0.08799
16	*NSKH*	0.02155	*NSmin*	- 0.133255	*NPPV*	0.03331
17	*NHmax*	- 0.01758	*HPP*	- 0.004589	*NP/cm*	0.02315
Cumul. Prop. *Kumul. prop.*		0.748		0.962		1.00

Table 27 shows the variables classified by each of the discriminant functions in order of the contribution to group discrimination. Together with the variables, there are the corresponding standardised coefficients.

The discrimination defined by discriminant function 1 is mostly determined by variable *DCC*, which is followed in turns by *DPP*, *NZ/cm*, *HPP*, *NSKM*, *NP/cm*, etc. The discriminant function 2 is mostly determined by variables *DPP*, *PPP*, *LT*, *LI*, *NPPV*, *NSmax*, etc., while the discriminant function 3 is determined by variables *DPP*, *PPP*, *DCC*, *HHPP*, *LI*, *NPPU*, etc. The last row in Table 55 shows a cumulative proportion of the explained variance of each function; The first function calculates 75% of the explained variance, which means that 75% of discrimination has been explained by this function. By adding the second function, i.e. the additional 21%, 96% of the explained variance has been calculated; the contribution of the third function is the smallest, the remaining 4%.

Table 28 shows the means of the variables of the four analysed groups, for each discriminant function.

Table 28. Means of variables for three discriminant functions, per groups

Tablica 28. Sredine kanonskih varijabla za tri diskriminacijske funkcije po grupama

Group *Grupa*	Discr. Function 1 *Diskr. funkcija 1*	Discr. Function 2 *Diskr. funkcija 2*	Discr. Function 3 *Diskr. funkcija 3*
ni	-1.84827	- 0.939939	- 0.256285
th	4.88996	- 0.179911	- 0.342686
nith	0.41042	- 0.200898	1.31566
thni	- 1.21275	2.979266	- 0.160081

The first discriminant function distinguishes best the Japanese black pine and the European black pine; it distinguishes very well the Japanese black pine from hybrids *thni*, and a bit less well the Japanese black pine from hybrids *nith* (Table 28). The means of F_1 *nith* and *thni* are relatively close to one another and are situated between the means of the parent species, though slightly closer to the European black pine. This is also seen in Figure 3, where the individual values of the first function, per group, are shown on the x-coordinate.

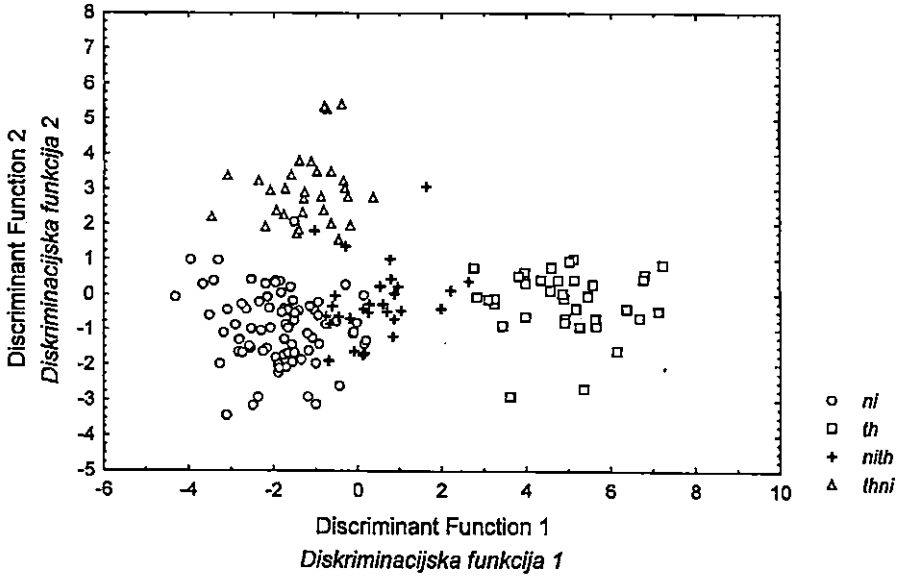

Figure 3. Graph of discriminant functions 1 and 2. Particular values for the first discriminant function are plotted on axis x, and for the second discriminant function on axis y.

Slika 3. Grafički prikaz diskriminacijskih funkcija 1 i 2. Na osi x su pojedinačne vrijednosti za prvu diskriminacijsku funkciju, a na osi y za drugu diskriminacijsku funkciju.

The second discriminant function distinguishes best F_1 hybrids *thni* from European black pine (Table 28). This function is graphically presented in Figure 3, coordinate y.

The third discriminant function includes only 4% of the explained variance.

The coefficients of the classification functions per group are shown in Table 29.

Table 29. Coefficients of classification functions, per groups

Tablica 29. Koeficijenti klasifikacijskih funkcija po grupama

Variable *Varijabla*	*ni*	*th*	*nith*	*thni*
NZ/cm	- 0.168	0.645	0.012	- 0.207
LT	21.712	37.411	27.031	35.461
NP/cm	1.798	1.467	1.707	1.892
NPPV	4.66	5.445	5.048	5.493
LI	1.344	0.753	0.729	0.558
DCC	- 133.897	- 219.258	- 182.207	- 132.453
HPP	386.815	417.475	410.662	390.451
NSmax	3.763	3.547	3.84	3.108
DPP	621.539	658.209	656.175	603.531
NSKM	- 2.028	- 3.324	- 2.546	- 1.655
NPPU	- 0.553	- 1.995	- 1.526	0.222
NSmin	1.866	0.087	1.107	1.285
LR	25.51	35.027	32.909	30.965
NSKH	8.944	9.447	10.064	10.628
PPP	- 593.355	- 593.573	- 606.247	- 580.601
DT	2.861	2.964	3.107	3.043
NHmax	16.451	16.042	15.399	15.081
Constant *Konstanta*	- 521.205	- 544.912	- 541.232	- 544.958

Classification matrix (Table 30) shows how good is the prediction of the new sample classification to the individual groups.

Table 30. Classification matrix

Tablica 30. Klasifikacijska matrica

Group *Grupa*	Percent Correct *Točno klasific.* %	*ni*		*th*		*nith*		*thni*		Total *Ukupno*
		%	No. *kom.*	%	No. *kom.*	%	%	No. *kom.*	%	No. *kom.*
ni	97.6	80	97.6	0	0	1	1.2	1	1.2	82
th	100	0	0	36	100	0	0	0	0	36
nith	73.3	5	16.7	1	3.3	22	73.3	2	6.7	30
thni	96.7	0	0	0	0	1	3.3	29	96.7	30

Based on seventeen analysed characteristics, a safe sample classification of the Japanese black pine is possible. There is a possibility of confusing the European black pine with hybrids *nith* in 1.2% of cases, and with hybrids *thni* in 1.2% of cases, i.e. the accuracy of classification is 97.6%, which is statistically significant (level 0.05).

The analysis was done to establish a possibility of discriminating the hybrids from their parent species. With hybrids *thni*, the accuracy of sample classification is satisfactory

(96.7%). There is a possibility of mistaking them for samples *nith* in 3.3% of cases. According to the analysed characteristics, hybrids *thni* can be perfectly discriminated from their parent species, the Japanese black pine and the European black pine.

The discrimination of hybrids *nith* from other groups on the basis of the analysed traits is impossible, since the accuracy of classifying their samples is only 73.3%. The highest probability of confusing them is with samples of the European black pine (16.7%), then with the samples of the *thni* hybrid (6.7%), and with the Japanese black pine (3.3%).

By comparing hybrids *nith* only with the parent species, the accuracy of hybrid *nith* classification has risen to 80%, which still does not make it significant (Table 31). This means that some other characteristics must be included into the analysis to discriminate hybrids *nith* from the parent species.

Table 31. Classification matrix

Tablica 31. Klasifikacijska matrica

Group *Grupa*	Percent Correct *Točno klasific.* %	*ni*		*th*		*nith*		Total *Ukupno*
		No. *kom.*	%	No. *kom.*	%	No. *kom.*	%	No. *kom.*
ni	98.8	81	98.8	0	0	1	1.2	82
th	100	0	0	36	100	0	0	36
nith	80.0	5	16.7	1	3.3	24	80	30

By comparing F_1 hybrids *thni* only with the parent species, the accuracy of classification has risen to 100% (Table 32), i.e. hybrids *thni*, based on the seventeen analysed characteristics, can be perfectly discriminated from the Japanese black pine and the European black pine.

Table 32. Classification matrix

Tablica 32. Klasifikacijska matrica

Group *Grupa*	Percent Correct *Točno klasific.* %	*ni*		*th*		*thni*		Total *Ukupno*
		No. *kom.*	%	No. *kom.*	%	No. *kom.*	%	No. *kom.*
ni	98.8	81	98.8	0	0	1	1.2	82
th	100	0	0	36	100	0	0	36
thni	100	0	0	0	0	30	100	30

THE RESULTS OF NEEDLE VOLATILES COMPOSITION ANALYSIS
REZULTATI ANALIZE SASTAVA ETERIČNIH ULJA IGLICA

We used fresh needles of each of the analysed pine species and hybrids to distill volatile oils. Using the previously described procedure, the volatile oils were analysed by using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The results of gas chromatography were chromatograms, both graphical and numerical. Each

chromatogram (of three species and four hybrids) was compared to all other chromatograms, in order to establish the concurrence of the individual components of the analysed groups. This concurrence was also tested graphically (by visual overlapping of the deviation on chromatograms) and numerically (by comparing the linear indices).

The linear indices are calculated in the following way:

$$I_x = 100\,z + 100\,[(t_R)_x - (t_R)_z] / [(t_R)_{z+1} - (t_R)_z]$$

x = component

t_R = total retention time

z = number of C atoms of the previous n-alcane standard

$z+1$ = number of C atoms of the following n-alcane standard

The numerical data of the chromatogram were used to make a quantitative analysis of the single components. The area below the component deviation, calculated by the integrator, is proportional to the concentration of the sample component. This means that for each component the quantification of the analysed components was made.

To give names to the components, that is in order to determine them in terms of quality, the GC/MS analysis was used, a method enabling a direct identification of the volatile components based on the spectrum of their mass. This analysis relates to computer scanning of the known mass spectrum components database.

With the compilation of the GC and GC/MS data, tables are formed for each of the species and hybrids, including the identified components and the percentages of these components related to the the total contents of the samples.

Altogether there are 131 components (detected components); some components are present in all analysed species and hybrids, others just in a smaller number of groups; fifty-five components were identified. The different proportions of the identified components in the total content of the volatile depended on the species and hybrids (Tables 34 and 35). The detected components of all groups together were processed using *cluster* analysis in order to determine which groups to what extent are similar in terms of needle volatile composition. The input data for cluster analysis were the areas below the chromatogram peaks, calculated as quantified components in relation to the total area calculated by an integrator.

Table 33 shows a numerical position of the node, that is the linkage distance of groups in clusters. The table shows, same as the dendrogram in Figure 4, that hybrids *nith* and *thni* are the most similar as to the volatile composition (4.7). They are followed by groups *ni - nide* (8.2) and *de - deni* (11.8). The cluster *nith - thni* has a linkage distance of 19.1 with group *th*, while groups *ni - nide* and *de - deni* are linked in the cluster at a distance of 26.0. Eventually, all groups are linked at a distance of 34.8.

Table 33. Linkage distances of groups in clusters

Tablica 33. Udaljenosti povezivanja grupa u clustere

Linkage Distance *Udaljenost povez.*	1	2	3	4	5	6	7
4.7	*nith*	*thni*					
8.2	*ni*	*nide*					
11.8	*de*	*deni*					
19.1	*th*	*nith*	*thni*				
26.0	*ni*	*nide*	*de*	*deni*			
34.8	*ni*	*nide*	*de*	*deni*	*th*	*nith*	*thni*

The linkage of the individual groups into clusters is logical. Figure 4 shows that the Japanese black pine and the hybrids to which this species is one of the parents (*nith* and *thni*) form one separate group. Another group is composed of two units. The first is composed of the European black pine and the hybrids *nide*, to which this species is the female parent, while the second unit contains the Japanese red pine and the hybrids *deni*, to which this species is also the female parent.

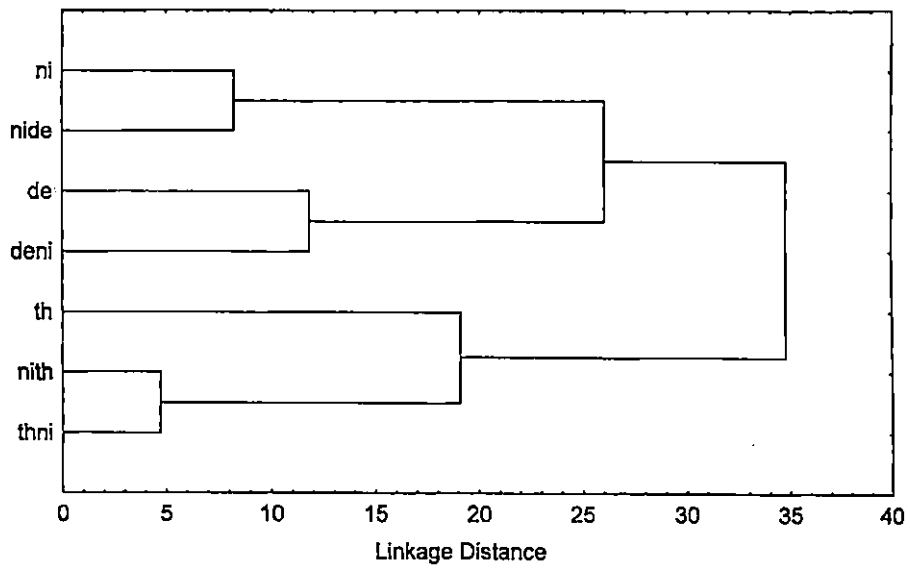

Figure 4. Horizontal hierarchical tree for seven analysed groups

Slika 4. Horizontalno hijerarhijsko stablo za sedam analiziranih grupa

The composition of the volatile is shown in two separate units, that is in two analyses. The first compares groups *ni*, *de*, *nide* and *deni*, while the second compares groups *ni*, *th*, *nith* and *thni*.

First analysis: *ni, de, nide, deni*
Prva analiza: *ni, de, nide, deni*

The needle volatile composition of the European black pine, the Japanese red pine and their F_1 hybrids *nide* and *deni* were analysed. The number of the detected components differs as to the the species and hybrids. The black pine and the Japanese red pine had 122 detected components each; the F_1 hybrids *nide* had 124 detected components, while in the reciprocal hybrids *deni* 114 components were found. Table 34 shows the identified components. In all four analysed groups, fifty-three components were identified. In the total content of the needle volatile of the European black pine, the proportion of the identified components is 94.4%. The respective proportions of the Japanese red pine, the F_1 hybrids *nide* and the reciprocal hybrids *deni* were 81.33%, 93.25%, and 88.36%. The remaining contents to 100% are the components that could not be reliably identified, and were found in small quantities.

The Japanese red pine and hybrids *deni* had a lower percentage of the identified components in the total content of the needle volatile. The reason for this was the fact that several components, some of which participated with more than 3% in the total content of the volatile, could not be identified. These were the components with linear indices higher than 2000. The same components were found either in very small quantities or in traces.

For the components that show deviations on the chromatogram - though not numerically registered, as they are found in very small quantities - there is a note in the table that they occur in traces.

Figures 5 - 8 are graphical illustrations of the components by histograms; the components, at least in one group, make a proportion higher than 10% (Figure 5); higher than 5% (Figure 6); 3% (Figure 7), and 1% (Figure 8).

In all four analysed groups, the highest proportion of all components is the proportion of α-pinene (*ni* 42.66%, *de* 25.78%, *nide* 40.55%, and *deni* 26.88%). As to the content of this monoterpene, the hybrids resemble the female parent, that is *nide* hybrids resemble the black pine, while *deni* hybrids resemble the Japanese red pine (Figure 5). The same applies to the content of β-pinene (*ni* 11.64%, *de* 5.78%, *nide* 10.93%, and *deni* 3.58%), and the content of β-phellandrene + limonene (*ni* 3.66%, *de* 10.68%, *nide* 4.73%, and *deni* 12.2%). β-phellandrene + limonene are registered on the chromatogram as one peak, while two components were detected by GC/MS method. They were all shown together, since it is impossible to express them separately in terms of quantity. According to the content of germacrene D (Figure 5), the hybrids are intermediary between the parent species (*ni* 17.72%, *de* 4.49%, *nide* 12.46%, and *deni* 13.95%).

Table 34. Volatile compounds in needle oil of Pinus nigra (= *ni*), P. densiflora (= *de*), and their F_1 hybrids (= *nide and deni*). Linear retention indices on the apolar column and quantification for all compounds are shown.

Tablica 34. Sastav eteričnih ulja iglica Pinus nigra (= ni), P. densiflora (= de), i njihovih F_1 hibrida (= nide i deni). Za identificirane je komponente naveden postotni udio u ukupnom sadržaju eteričnih ulja i linearni retencijski indeksi na nepolarnoj koloni.

Peak *Vrh*	Compound *Komponenta*	*ni*		*de*		*nide*		*deni*	
		%	Index *Indeks*	%	Index *Indeks*	%	Index *Indeks*	%	Index *Indeks*
1	trans-2-hexenal	0.1	835	0.1	835	0.08	829	0.12	834
2	tricyclene	0.19	920	0.9	918	0.72	915	0.79	919
3	α-thujene	0.28	925	0.05	922	0.14	920	0.15	924
4	α-pinene	42.66	935	25.78	930	40.55	930	26.88	932
5	camphene	1.13	943	3.41	940	2.65	938	3.02	942
6	sabinene	traces		0.37	963	0.25	961	0.45	965
7	β-pinene	11.64	971	5.78	967	10.93	966	3.58	968
8	myrcene	1.39	986	8.01	983	4.46	981	6.94	986
9	α-phellandrene	0.06	998	0.09	995	0.06	993	0.12	997
10	Δ-3-carene	0.2	1005	0.03	1002	0.04	1000	0.02	1005
11	α-terpinene	0.06	1010	0.08	1007	0.05	1005	0.06	1009
12	p-cymene	0.02	1014	0.02	1011	0.01	1009	traces	
13	β-phellandrene + limonene	3.66	1022	10.68	1018	4.73	1016	12.2	1021
14	cis-ocimene	0.02	1030	traces		0.02	1025	0.02	1029
15	trans-β-ocimene	1.03	1041	traces		1.09	1036	1.23	1040
16	γ-terpinene	0.05	1050	0.11	1046	0.06	1045	0.08	1048
17	α-terpinolene	0.61	1079	3.06	1076	2.13	1075	2.64	1078
18	linalool	0.06	1088	0.04	1085	0.04	1083	0.02	1088
19	α-campholene aldehyde	0.03	1107	0.03	1105	0.01	1104	0.02	1107
20	camphor	0.05	1122	0.02	1122	0.03	1118	0.01	1121
21	borneol	0.05	1148	0.05	1145	0.07	1144	0.11	1147
22	terpinen-4-ol	0.05	1161	0.09	1158	0.05	1157	0.06	1160
23	α-terpineol	0.19	1173	0.15	1170	0.15	1169	0.07	1172
24	methyl thymylether	0.04	1218	0.18	1215	0.06	1214	0.18	1217
25	linalyl acetate	0.22	1244	traces		0.03	1240	0.03	1243
26	bornyl acetate	0.81	1269	2.62	1267	3.18	1267	4.98	1269
27	α-terpinyl acetate	0.37	1333	0		0.07	1329	0.02	1332
28	bicycloelemene	0.02	1343	0.02	1340	0.02	1340	0.02	1342
29	geranyl acetate	0.01	1364	0.15	1363	0.09	1363	0.08	1365
30	α-copaene	0.07	1368	0.03	1365	0.06	1365	0.07	1367
31	β-bourbonene	0.23	1378	0		0.19	1374	0.13	1376
32	β-elemene	0.02	1383	0.05	1380	0.03	1379	0.1	1380
33	β-caryophyllene	5.62	1411	3.77	1406	4.05	1407	5.28	1409
34	β-cubebene	0.03	1418	traces		0.04	1415	0.03	1417
35	aromadendrene	traces		0.03	1425	0.02	1425	traces	
36	α-humulene	0.9	1442	0.59	1438	0.63	1438	0.8	1441

Peak Vrh	Compound *Komponenta*	*ni*		*de*		*nide*		*deni*	
		%	Index *Indeks*	%	Index *Indeks*	%	Index *Indeks*	%	Index *Indeks*
37	sesquiterpene hydrocarbon (M 204)	0.07	1450	0.04	1445	0.05	1447	0.06	1449
38	γ-muurolene	0		0.16	1462	0		0	
39	germacrene D	17.72	1474	4.49	1466	12.46	1470	13.95	1471
40	α-muurolene	0.26	1484	0.84	1481	0.39	1481	0.3	1483
41	β-cadinene	0.03	1494	0.03	1491	0.03	1491	0.04	1493
42	γ-cadinene	0.25	1500	0.33	1497	0.27	1497	0.27	1499
43	δ-cadinene	0.48	1510	0.61	1507	0.47	1507	0.43	1509
44	4,10-dimethyl-7-isopropyl (4,4,0)-bicyclo -1,4-decadiene	0.01	1518	traces		0.01	1515	0.01	1517
45	α-cadinene	0.03	1524	0.04	1521	0.03	1520	0.03	1523
46	endo-1-bourbonanol	0.58	1558	1.03	1555	0.56	1555	0.4	1556
47	caryophyllene oxide	0.1	1561	0.05	1558	0.03	1558	0.05	1560
48	oxygenated sesquiterpene (M 222)	0.02	1608	0.05	1606	0.02	1605	0.01	1607
49	α-cadinol	0.23	1621	0.57	1618	0.21	1618	0.18	1619
50	sesquiterpene hydrocarbon (M 204)	0.05	1624	0.14	1621	0.06	1621	0.04	1623
51	T-muurolol	0.27	1633	0.75	1630	0.25	1630	0.2	1631
52	oxygenated sesquiterpene (M 220)	0.09	1662	0.03	1659	0.03	1658	0.04	1660
53	13-epimanoyl oxyde	2.25	1973	0.32	1969	0.09	1968	0.07	1969
54	thunbergol	0.09	2031	5.56	2032	1.5	2030	1.97	2031
Σ		94.4		81.33		93.25		88.36	

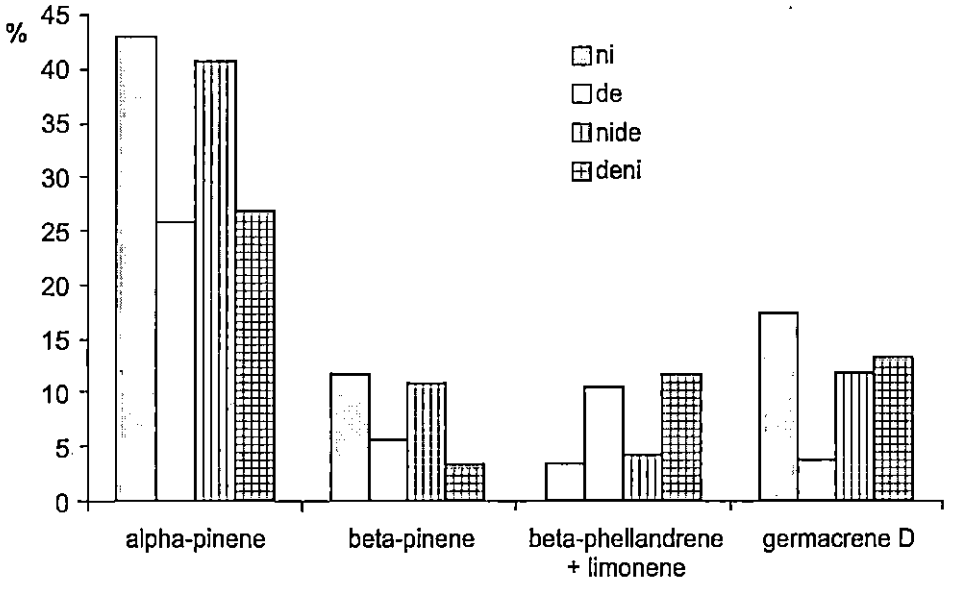

Figure 5. Components with more than 10 % of the total essential needle oil in at least one group (species or hybrid)

Slika 5. Komponente kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu (vrstu ili hibrid) više od 10 %

As to the content of myrcene (Figure 6), hybrids *nide* are intermediary between the parent species, while hybrids *deni* are closer to the female parent, the Japanese red pine (*ni* 1.39%, *de* 8.01%, *nide* 4.46%, and *deni* 6.94%).

The proportion of β-caryophyllene (Figure 6) in hybrids *nide* is closer to the Japanese red pine, while in hybrids *deni* it is closer to the black pine (*ni* 5.62%, *de* 3.77%, *nide* 4.05%, and *deni* 5.28%).

Thunbergol is a diterpene characteristic of the analysed Japanese pine species (*P. densiflora* and *P. thunbergiana*), while in the European species (*P. nigra* and *P. sylvestris*), it occurs either in very small quantities or in traces (Figure 6). Hybrids *nide* and *deni* are, as to the content of thunbergol, intermediary between the parent species, though closer to the black pine (*ni* 0.09%, *de* 5.56%, *nide* 1.5%, and *deni* 1.97%).

The proportion of camphene and α-terpinolene in both hybrid combinations is intermediary between the parent species (Figure 7), while with hybrid *deni* it is closer to the Japanese red pine (camphene: *ni* 1.13%, *de* 3.41%, *nide* 2.65, *deni* 3.02%; α-terpinolene: *ni* 0.61%, *de* 3.06%, *nide* 2.13, *deni* 2.64%).

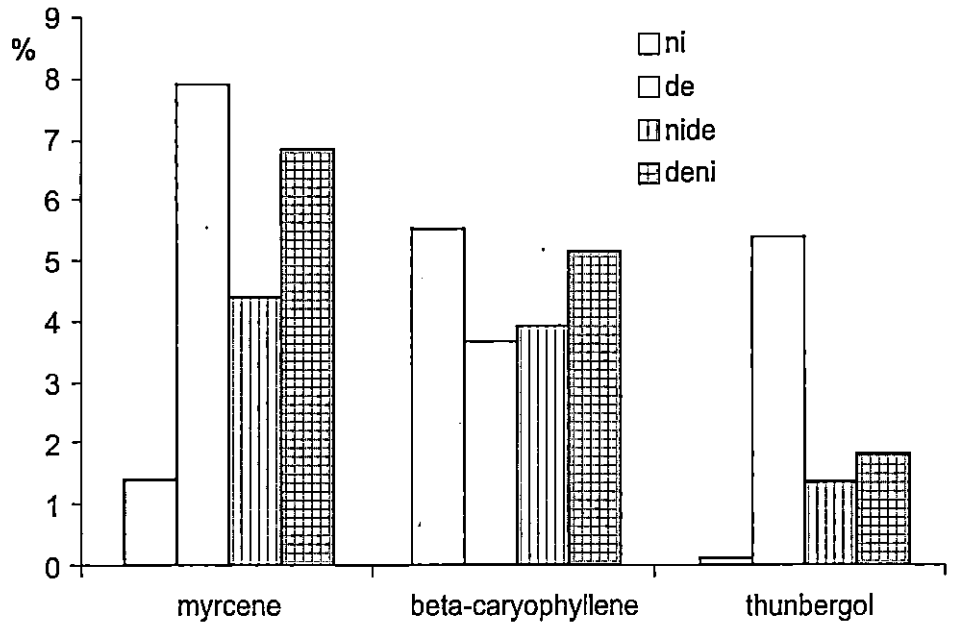

Figure 6. Components with more than 5 % of the total essential needle oil in at least one group

Slika 6. Terpeni kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu više od 5 %

Bornyl acetate (Figure 7) has a higher proportion in hybrid volatile than in the volatile of the parent species (*ni* 0.81%, *de* 2.62%, *nide* 3.18%, *deni* 4.98%).

The proportion of *trans*-β-ocimene (Figure 8) is slightly higher in hybrids than in black pine, while in the Japanese red pine it is present only in traces (*ni* 1.03%, *de* traces, *nide* 1.09%, *deni* 1.23%).

The proportions of sesquiterpene *endo*-1-bourbonanol (Figure 8) in black pine and hybrid *nide* are similar (*ni* 0.58%, *nide* 0.56%); the highest proportion was in the Japanese red pine (1.03%), and the lowest proportion was found in hybrid *deni* (0.4%).

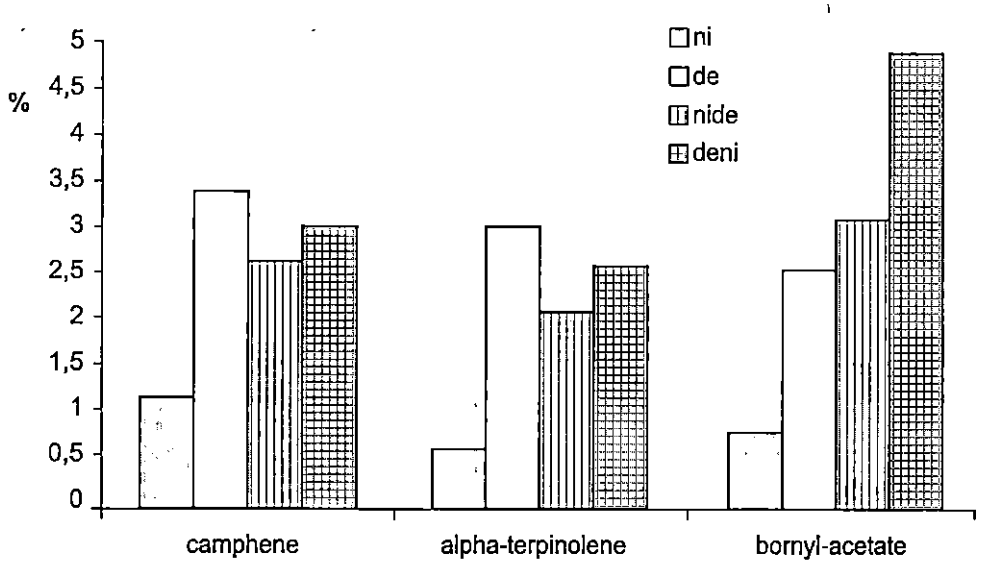

Figure 7. Components with more than 3 % of the total essential needle oil in at least one group

Slika 7. Terpeni kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu više od 3 %

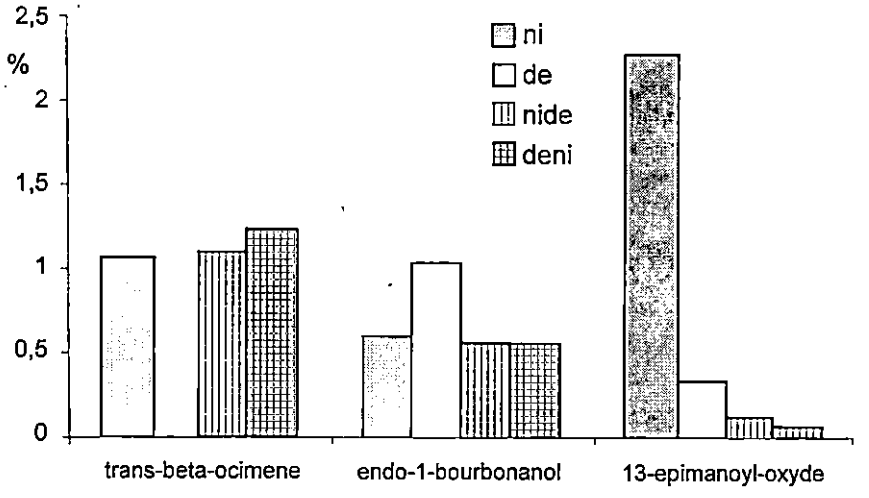

Figure 8. Components with more than 1 % of the total essential needle oil in at least one group

Slika 8. Terpeni kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu više od 1 %

The proportion of 13-epimanoyl oxyde (Figure 8) is in the black pine higher than in the Japanese red pine (*ni* 2.25%, *de* 0.32%), while the hybrids have a smaller proportion of this diterpene than their parent species (*nide* 0.09%, *deni* 0.07%).

F_1 hybrids *P. nigra* × *P. densiflora*
F_1 hibridi *P. nigra* × *P. densiflora*

Of the 124 detected components fifty-three were identified. The identified components make 93.25% of the total needle volatile. All identified components are terpenes (93.17%), except for the *trans*-2-hexenal (0.08%), which is an n-alkene (peak 1 in Table 34).

The largest number, twenty-eight, of the identified components are monoterpenes (peaks 2 - 29 in Table 34). They participate in the total content of the volatile with 71.69%, which is more than both parent species (*nide* 64.9%, *deni* 61.72%). Monoterpenes with the highest proportion in the needle volatile content are α-pinene (40.55%), β-pinene (10.93%), β-phellandrene + limonene (4.73%) and myrcene (4.46%). As to the proportion of α-pinene and β-pinene, hybrids *nide* are similar to the black pine (α-pinene: *ni* 42.66%, *de* 25.78%; β-pinene: *ni* 11.64%, *de* 5.78%). The proportion of β-phellandrene + limonene and the proportion of myrcene is in the hybrids intermediary between the parent species (Figures 5 and 6).

There are twenty-two sesquiterpenes (19.89%) (peaks 30 - 37, 39 - 52 in Table 34), of which eighteen were identified (19.73%) and four partly described (0.16%). The main components in this group of compounds are germacrene D (12.46%) and β-caryophyllene (4.05%). According to the proportions of these two components, the hybrids are intermediary between their parent species (germacrene D: *ni* 17.72%, *de* 4.49%; β-caryopyllene: *ni* 5.62%, *de* 3.77%) (Figures 5 and 6).

Two diterpenes were identified, 13-epimanoyl oxide (0.09%) and thunbergol (1.5%) (peaks 53 and 54 in Table 34).

F_1 hybrids *P. densiflora* × *P. nigra*
F_1 hibridi *P. densiflora* × *P. nigra*

Fifty-three components were identified in the 114 detected components of hybrids *deni*. The proportion of the identified components is 88.36% of the needle volatile content: 52 components are terpenes (88.24%), while one component, *trans*-2-hexenal (0.12%), is n-alkene (peak 1 in Table 34).

Monoterpenes are the main group of compounds, altogether twenty-eight (peaks 2 - 29 in Table 34). The proportion of monoterpenes in the total content of the needle volatile is 63.78%, which makes hybrids *deni* intermediary between the parent species (*ni* 64.9%, *de* 61.72%). In this group of compounds, the most significant components are α-pinene (26.88%) and β-pinene (3.58%). As to the proportion of α-pinene, β-phellandrene + limonene, myrcene and β-pinene, hybrids *deni* resemble the Japanese red pine (α-pinene: *ni* 42.66%, *de* 25.78%; β-phellandrene + limonene: *ni* 3.66%, *de* 10-68%; myrcene: *ni* 1.39%, *de* 8.01%; β-pinene:

ni 11.64%, *de* 5.78%). The proportion of bornyl acetate is higher in the hybrids than in the parent species, though closer to the Japanese red pine (*ni* 0.81%, *de* 2.62%) (Figures 5 7).

Next in significance, sesquiterpenes are the group of compounds twenty-two in number (peaks 30 - 37; 39 - 52 in Table 34). Eighteen sesquiterpenes were identified (22.27%), while four were partly described (0.15%). The most significant components in this compound group are germacrene D (13.95%) and β-caryophyllene (5.28%). The proportion of these components in hybrids *deni* is intermediary between the parent species (germacrene D: *ni* 17.72%, *de* 4.49%; β-caryophyllene: *ni* 5.62%, *de* 3.77%) (Figures 5 and 6).

The identified diterpenes are 13-epimanoyl oxide (0.07%) and thunbergol (1.97%).

Second analysis: *ni, th, nith, thni*
Druga analiza: *ni, th, nith, thni*

The needle volatile composition of the European black pine, the Japanese black pine and their F_1 hybrids *nith* and *thni* were analysed. There were 122 detected components of the European black pine and 87 components of the Japanese black pine; F_1 hybrids *nith* had 120 detected components, while 78 components were found in the reciprocal hybrids *thni*. Table 35 shows the identified components.

Fifty-three components were identified for the European black pine; 50 components of the Japanese black pine; 53 components of hybrids *nith*, and 42 components of hybrids *thni*. Of the total content of the needle volatile of the European black pine, 94.4% were identified components. The respective proportions of the Japanese black pine, F_1 hybrids *nith* and reciprocal hybrids *thni* are 94.85%, 95.99% and 96.1%.

Figures 9 - 11 present graphically in histograms the component which, at least in one group, make the proportion higher than 10% (Figure 9); higher than 3% (Figure 10), and higher than 1% (Figure 11).

Table 35. Volatile compounds in needle oil of of Pinus nigra (= *ni*), P. thunbergiana (= *th*), and their F_1 hybrids (= *nith and thni*). Linear retention indices on the apolar column and quantification for all compounds are shown.

Tablica 35. Sastav eteričnih ulja iglica Pinus nigra (= ni*), P. thunbergiana (=* th*), i njihovih F_1 hibrida (=* nith i thni*). Za identificirane je komponente naveden postotni udio u ukupnom sadržaju eteričnih ulja i linearni retencijski indeks na nepolarnoj koloni.*

Peak *Vrh*	Compound *Komponenta*	*ni*		*th*		*nith*		*thni*	
		%	Index *Indeks*	%	Index *Indeks*	%	Index *Indeks*	%	Index *Indeks*
1	trans-2-hexenal	0.1	835	0.49	832	0.52	829	0.18	830
2	tricyclene	0.19	920	0.44	918	0.38	916	0.62	916
3	α-thujene	0.28	925	0.04	923	0.25	921	0.36	920
4	α-pinene	42.66	935	19.56	929	31	930	30.63	928
5	camphene	1.13	943	1.8	940	1.76	939	2.49	938
6	sabinene	traces		traces		traces		0.36	962
7	β-pinene	11.64	971	34.13	969	26.45	969	22.95	966
8	myrcene	1.39	986	4.7	984	2.28	982	2.75	981
9	α-phellandrene	0.06	998	0.12	996	0.08	994	0.08	993
10	Δ-3-carene	0.2	1005	0.03	1004	0.05	1001	0.12	1001
11	α-terpinene	0.06	1010	0.05	1008	0.06	1006	0.08	1006
12	p-cymene	0.02	1014	traces		0.01	1010	0.02	1014
13	β-phellandrene + limonene	3.66	1022	10.56	1019	7.99	1018	4.69	1016
14	cis-ocimene	0.02	1030	traces		0.02	1026	0.03	1026
15	trans-β-ocimene	1.03	1041	traces		0.7	1037	0.5	1036
16	γ-terpinene	0.05	1050	0.08	1047	0.07	1045	0.08	1045
17	α-terpinolene	0.61	1079	2.64	1077	1.83	1076	3.4	1075
18	linalool	0.06	1088	0.06	1086	0.03	1084	0	
19	α-campholene aldehyde	0.03	1107	0.03	1106	0.03	1104	0	
20	camphor	0.05	1122	0.03	1121	0.03	1118	0	
21	borneol	0.05	1148	0.06	1147	0.06	1144	0.05	1145
22	terpinen-4-ol	0.05	1161	0.08	1159	0.08	1157	0.06	1157
23	α-terpineol	0.19	1173	0.45	1171	0.35	1169	0.21	1169
24	methyl thymylether	0.04	1218	0.18	1216	0.11	1215	0.06	1215
25	linalyl acetate	0.22	1244	traces		0.03	1241	0	
26	bornyl acetate	0.81	1269	1.94	1267	2.29	1267	3.13	1266
27	α-terpinyl acetate	0.37	1333	0		0.28	1330	0	
28	bicycloelemene	0.02	1343	0.03	1341	0.01	1340	0	
29	geranyl acetate	0.01	1364	0.25	1364	0.05	1363	0	

Peak *Vrh*	Compound *Komponenta*	*ni*		*th*		*nith*		*thni*	
		%	Index *Indeks*	%	Index Indeks	%	Index *Indeks*	%	Index *Indeks*
37	sesquiterpene hydrocarbon (M 204)	0.07	1450	0.03	1446	0.04	1447	0.07	1446
38	γ-muurolene	0		0.29	1463	0		0	
39	germacrene D	17.72	1474	8.33	1467	11.83	1470	13.63	1467
40	α-muurolene	0.26	1484	0.71	1481	0.34	1481	0.55	1480
41	β-cadinene	0.03	1494	0.06	1492	0.03	1491	0.02	1491
42	γ-cadinene	0.25	1500	0.47	1497	0.21	1497	0.26	1496
43	δ-cadinene	0.48	1510	0.82	1507	0.42	1507	0.51	1506
44	4,10-dimethyl-7-isopropyl (4,4,0)-bicyclo-1,4-decadiene	0.01	1518	0		0.01	1515	0	
45	α-cadinene	0.03	1524	0.04	1521	0.03	1521	0.03	1520
46	endo-1-bourbonanol	0.58	1558	0.66	1555	0.66	1555	1.14	1554
47	caryophyllene oxide	0.1	1561	traces		0.04	1558	0	
48	oxygenated sesquiterpene (M 222)	0.02	1608	0		0.02	1605	0.02	1604
49	α-cadinol	0.23	1621	0.3	1618	0.25	1618	0.33	1617
50	sesquiterpene hydrocarbon (M 204)	0.05	1624	0.08	1621	0.05	1621	0.06	1620
51	T-muurolol	0.27	1633	0.35	1630	0.32	1630	0.42	1629
52	oxygenated sesquiterpene (M 220)	0.09	1662	0.04	1658	0.04	1658	0	
53	13-epimanoyl oxyde	2.25	1973	0.07	1968	0.05	1967	0.04	1967
54	thunbergol	0.09	2031	0.67	2027	0.55	2027	1.53	2027
Σ		94.4		94.85		95.99		96.1	

The proportions of the four components shown in Figure 9 are in both hybrid cross-breeding combinations intermediary between the parent species (α-pinene: *ni* 42.66%, *th* 19.56%, *nith* 31.0%, *thni* 30.63%; β-pinene: *ni* 11.64%, *th* 34.13%, *nith* 26.45, *thni* 22.95%; β-phellandrene + limonene: *ni* 3.66%, *th* 10.56%, *nith* 7.99, *thni* 4.69%; germacrene D: *ni* 17.72%, *th* 8.33%, *nith* 11.83, *thni* 13.63%). The component, which has the highest percentage in the needle volatile of the European black pine and of hybrids *nith* and *thni*, is α-pinene, while the same applies to β-pinene in the Japanese black pine.

The proportion of myrcene in the needle volatile of the Japanese black pine is 4.7%. This proportion is 1.39% with the European black pine (Figure 10). The proportion of myrcene in the hybrids is intermediary between the parent species (*nide* 2.28%, *deni* 2.75%).

The proportion of bornyl acetate is higher in the needles of the hybrids than of the parent species (*ni* 0.81%, *th* 1.94%, *nith* 2.29, *thni* 3.13%). The proportion of this component in hybrids *nith* and *thni* is closer to the values of the Japanese black pine than those of the European black pine (Figure 10).

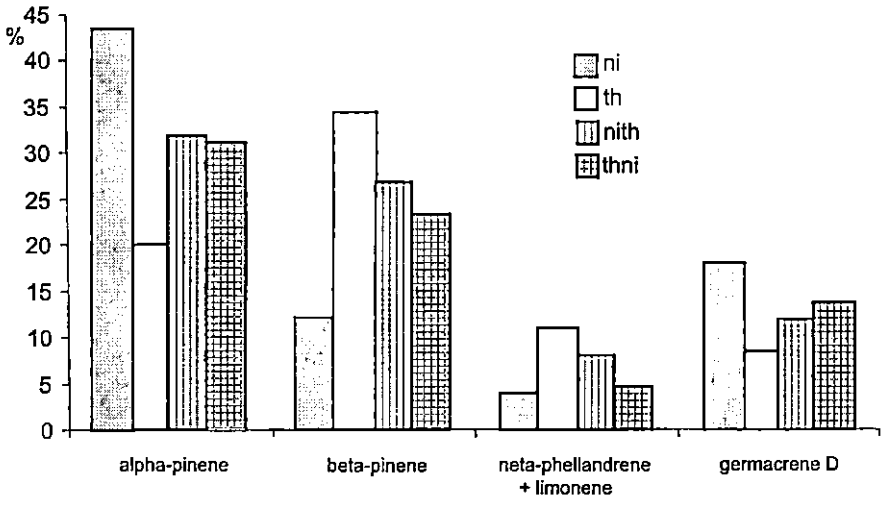

Figure 9. Components with more than 10 % of the total essential needle oil in at least one group

Slika 9. Terpeni kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu više od 10 %

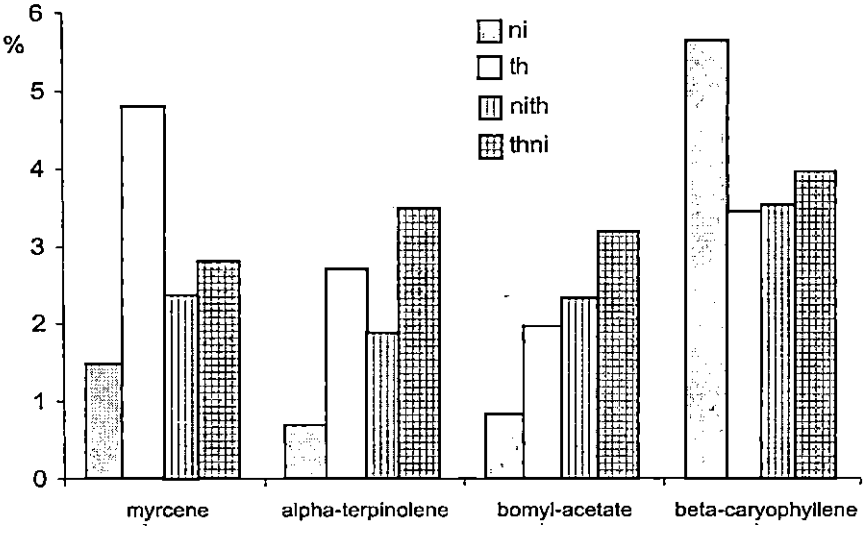

Figure 10. Components with more than 3 % of the total essential needle oil in at least one group

Slika 10. Terpeni kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu više od 3 %

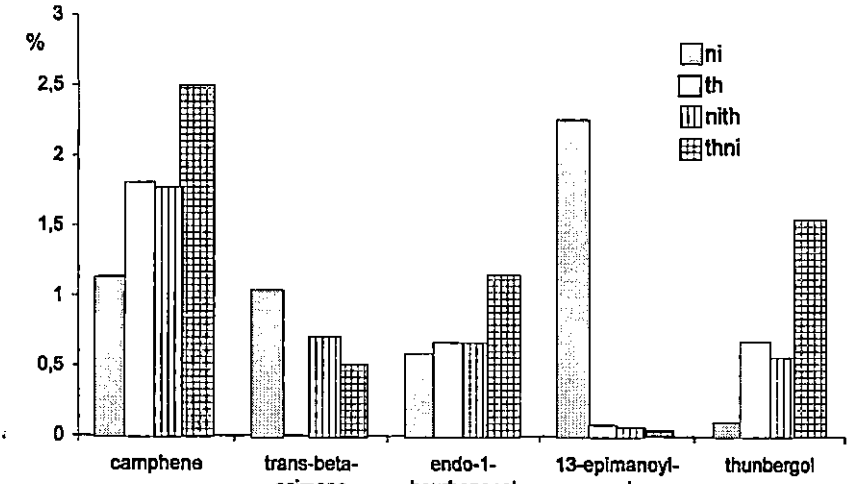

Figure 11. Components with more than 1 % of the total essential needle oil in at least one group

Slika 11. Terpeni kojih je u ukupnom sadržaju eteričnih ulja iglica za barem jednu grupu više od 1 %

Sesquiterpene β-caryophyllene (Figure 10) has the highest proportion in the needle volatile of the European black pine, while its proportions in the Japanese black pine and hybrids *nith* and *thni* are almost the same (*ni* 5.62%, *th* 3.42%, *nith* 3.5, *thni* 3.92%).

The proportion of camphene (Figure 11) is the lowest in the European black pine, the highest in hybrid *thni*, and almost the same in the Japanese black pine and hybrid *nith* (*ni* 1.13%, *th* 1.8%, *nith* 1.76, *thni* 2.49%). Similar proportions were found in sesquiterpene *endo*-1-bourbonanol and diterpene thunbergol (*endo*-1-bourbonanol: *ni* 0,58%, *th* 0.66%, *nith* 0.66, *thni* 1.14%; thunbergol: *ni* 0.09%, *th* 0.67%, *nith* 0.55, *thni* 1.53%).

The proportion of *trans*-β-ocimene in the needle volatile of the European black pine was 1.03%, while in the Japanese black pine it was found only in traces (Figure 11). The proportion of this monoterpene in hybrids is intermediary between the parent species (*nith* 0.07, *thni* 0.5%).

The proportion of diterpene 13-epimanoyl oxide (Figure 11) in the needle volatile of the European black pine is 2.25%, while a very small proportion is found in the Japanese black pine and hybrids *nith* and *thni* (*th* 0,07%, *nith* 0.05, *thni* 0.04%).

F_1 hybrids *P. nigra* × *P. thunbergiana*
F_1 hibridi *P. nigra* × *P. thunbergiana*

Of the 120 detected components, fifty-three were identified. The identified components

make 95.99% of the total needle volatile of hybrid *nith*. Fifty-two components are terpenes (95.47%), except the *trans*-2-hexenal (0.52%), which is an n-alkene (peak 1 in Table 35).

The largest number, twenty-eight, of the identified components are monoterpenes (peaks 2 - 29 in Table 35). The proportion of monoterpenes in the total content of needle volatile is 76.3%, which makes hybrids *nith* similar to the Japanese black pine (*ni* 64.9%, *th* 77.26%). The proportions of the monoterpenes α-pinene (31.0%), β-pinene (26.45%) and β-phellandrene + limonene (7.99%) are the highest. The proportion of α-pinene in hybrids *nith* is intermediary between the parent species (*ni* 42.66%, *th* 19.56%). As to the proportions of β-pinene and β-phellandrene + limonene, the hybrids are more similar to the Japanese black pine (β-pinene: *ni* 11.64%, *th* 34.13%; β-phellandrene + limonene: *ni* 3.66%, *th* 10.56%) (Figure 9).

Sesquiterpenes are the next group of compounds, twenty-two in number (peaks 30 - 37; 39 - 52 in Table 35). Eighteen sesquiterpenes were identified (18.44%), while four were partly described (0.15%). The most significant components in this compound group are germacrene D (11.83%) and β-caryophyllene (3.5%). As to the proportion of these components, hybrids *nith* are more similar to the Japanese black pine than to the European black pine (germacrene D: *ni* 17.72%, *th* 8.33%; β-caryophyllene: *ni* 5.62%, *th* 3.42%) (Figures 9 and 10).

In hybrids *nith*, two diterpenes were identified, 13-epimanoyl oxide (0.05%) and thunbergol (0.55%). As to the proportions of these components (Figure 11), the hybrids resemble more the Japanese pine (13-epimanoyl oxide: *ni* 2.25%, *th* 0.07%; thunbergol: *ni* 0.09%, *th* 0.67%).

F_1 hybrids *P. thunbergiana* × *P. nigra*
F_1 hibridi *P. thunbergiana* × *P. nigra*

In hybrids *thni*, seventy-eight components were detected. According to this number, these hybrids resemble the Japanese black pine, in which eighty-seven components were detected, while 122 components were found in the European black pine. Forty-two components were identified, fewer than in the parent species (*ni* 53, *th* 50). However, these components make 96.1% of the needle volatile content, which is more than with the parent species (*ni* 94.4%, *th* 94.85%). In the identified components, forty-one are terpenes (95.92%), while one component, *trans*-2-hexenal (0.18%), is an n-alkene (peak 1 in Table 35).

Monoterpenes are the most significant group of compounds. There are twenty-one monoterpenes (peaks 2 - 17, 21 - 24 and 26 in Table 35). Their proportion in the total content of the needle volatile is 72.7%, which makes hybrids *thni* and *nith* similar to the Japanese black pine (*ni* 64.9%, *th* 77.26%). The most significant components of this group are α-pinene (30.63%), β-pinene (22.95%) and β-phellandrene + limonene (4.69%). The proportions of α-pinene and β-pinene in hybrids *thni* are intermediary as to the respective proportions in the parent species (α-pinene: *ni* 42.66%, *th* 19.56%; β-pinene: *ni* 11.64%, *th* 34.13%). As to the proportion of β-phellandrene + limonene, the hybrids are more similar to the European black pine (*ni* 3.66%, *th* 10.56%) (Figure 9).

There are eighteen sesquiterpenes (peaks 30 - 34, 36, 37, 39 -43, 45, 46, 48 - 51, Table 35). Fifteen sesquiterpenes were identified (21.53%), while three were partly described (0.15%).

The most significant components in this compound group are germacrene D (13.63%) and β-caryophyllene (3.92%). The proportion of germacrene D in hybrids *thni* is intermediary between the parent species (*ni* 17.72%, *th* 8.33%). The proportion of β-caryophyllene makes these hybrids more similar to the Japanese black pine (*ni* 5.62%, *th* 3.42%) (Figures 9 and 10).

The identified diterpenes are 13-epimanoyl oxide (0.04%) and thunbergol (1.53%) (Figure 11).

DISCUSION
RASPRAVA

DESCRIPTIVE STATISTICS
DESKRIPTIVNA STATISTIKA

Average needles and tracheids of one-year shoots in the measured sample (Tables 2 - 20) can be described in the following way:

European black pine
Europski crni bor

The average lengths of the needles and the fascicle sheath are 12.4 cm and 1 cm respectively. In the middle of the needle length there are twenty ventral stomatal rows, of which number eight are ventral and twelve are dorsal. There are 101 stomata along one stomatal row on the inner side of the needle, on a 1 cm long segment from the middle of the needle. On the same segment, along one needle margin, there are thirty-two serrations. In the middle of the needle's cross-section area there is a central cylinder, the stellar region, whose area is 0.2724 mm^2, its height 0.427 mm, and its diameter 0.796. The largest average number of hypodermal cell layers on the needle cross-section is 3.4, which is more than in other analysed groups. The average number of resin canals is six, and all of them are located medially. Each resin canal is surrounded by a layer of epithelial cells surrounded by sheath cells. The number of sheath cells around the canal was counted on the cross-section parts with the smallest number of cells (8.3) and the largest number of cells (13.4).

The average lengths and diameters of one-year shoot tracheids are 1.055 mm and 24.1 μm respectively. .

Japanese red pine
Japanski crveni bor

The average needle length is 12.0. The fascicle sheath length is 1.0 cm. There are seven ventral stomatal rows and ten dorsal stomatal rows in the middle of the needle length. On a 1 cm long segment in the middle of the needle along the ventral stomatal rows, there are 119 stomata, more than in all other analysed species and hybrids. On the same segment, along

one needle rim, there are fifty-four serrations. The area of the needle cross-section in 0.5935 mm², its height 0.733 mm, and its diameter 1.135 mm. The stellar region cross-section area is 0.1479 mm²; its height is 0.319 mm, and its diameter 0.591 mm. The average cross-section area values of the needle and of the stellar region are smaller in the Japanese red pine (it has the thinnest needles) than in other analysed groups. On a single cross-section there is the largest average number of layers of hypodermal cells (1.3). The resin canals are located near the hypoderm. Their average number is 6.5 Of all analysed groups, the Japanese red pine is the only one that typically have all resin canals located near the hypoderm. The largest number of sheath cells around the resin canal is 11.6, the smallest is 7.3, which is fewer than with other analysed groups.

The average tracheid length and width of one-year shoots are 1.232 mm and 21.9 μm respectively.

Japanese black pine
Japanski crni bor

The average needle length is 12.7 cm. The fascicle sheath length is 1.1 cm. There are twenty ventral stomatal rows, seven ventral and thirteen dorsal. On a 1 cm long segment in the middle of the needle along the ventral stomatal rows, there are ninety-two stomata, which is the smallest average number of stomata in all analysed groups. On the same segment, along one needle rim, there are sixty serrations, which is more than in other analysed species and hybrids. The average area of the needle cross-section is 1.0056 mm², its height is 1.058 mm, and its diameter 1.379 mm. The average stellar region cross-section area is 0.2501 mm²; its height is 0.452 mm, and its diameter 0.704 mm. The average cross-section area values of the needle and of the stellar region of the Japanese black pine are the highest of all other analysed groups. The largest average number of layers of hypodermal cells is 3.1. The resin canals are medial, and their average number is 4.6. Of all analysed groups, the Japanese black pine has the smallest number of resin canals located in the middle along the needle length. The largest number of sheath cells around the resin canal is 11.3 - fewer than in other species and hybrids - while the smallest respective number is 7.7.

The average tracheid length and width of one-year shoots are 1.232 mm (the longest of all analysed groups) and 21.9 μm, respectively.

F_1 hybrids *nide*
F_1 hibridi *nide*

The average needle length is 13.0 cm, which means that hybrids *nide* have the longest needles of all analysed groups. The average fascicle sheath length is 1.1 cm. Altogether there are eighteen stomatal rows in the middle of the needle length, of which seven are ventral and eleven are dorsal. On a 1 cm long segment in the middle of the needle along the ventral stomatal rows, there are 104 stomata. On the same segment, along one needle rim, there are thirty-five serrations. The area of the needle cross-section is 1.8180 mm², its height is

0.873 mm, and its width 1.313 mm. The stellar region cross-section area is 0.2153 mm²; its height is 0.376 mm, and its diameter is 0.717 mm. The largest average number of layers of hypodermal cells on a cross-section is 1.3. Their average number of resin canals located near the hypoderm is 2.4. The average number of resin canals is 7.7, of which 5.6 are located medially, and 2.1 near the hypoderm. The largest number of sheath cells are located around the resin canals (13.9), while their smallest average number is 8.5.

The average tracheid length and width of one-year shoots are 1.065 mm and 21.1 μm respectively.

F_1 hybrids *deni*
F_1 hibridi *deni*

The average needle length is 11.5, while the average fascicle sheath length is 0.9 cm. In the middle of the needle length, there are eight ventral and eleven dorsal stomatal rows. On a 1 cm long segment in the middle of the needle along the ventral stomatal rows, there are 103 stomata. On the same segment, along one needle rim, there are forty serrations. The average area of the needle cross-section is 0.8142 mm², its height is 0.898 mm, and its width 1.308 mm. The stellar region cross-section area is 0.2250 mm²; its height is 0.384 mm, and its diameter 0.743 mm. The largest average number of layers of hypodermal cells on a cross-section is 2.5. The average number of resin canals located near the hypodermis is 2.4. The average number of resin canals is 7, of which 4.1 are located medially, and 2.9 near the hypoderm. The largest number of sheath cells are located around the resin canals (14.1), while their smallest average number is 8.8. This smallest number of cells is larger than in other analysed groups.

The average tracheid length and width of one-year shoots are 1.087 mm and 20.3 μm respectively. Hybrids *deni* have the narrowest tracheids of all analysed groups.

F_1 hybrids *nith*
F_1 hibridi *nith*

The average needle length is 10.2, while the average fascicle sheath length is 1.0 cm. In the middle of the needle length, there are seven ventral and twelve dorsal stomatal rows. On a 1 cm long segment in the middle of the needle along the ventral stomatal rows, there are ninety-nine stomata. On the same segment, along one needle rim, there are thirty-seven serrations. The average area of the needle cross-section is 0.9799 mm², its height is 0.998 mm, and its width 1.414 mm. The stellar region cross-section area is 0.2501 mm²; its height is 0.424 mm, and its diameter 0.745 mm. The largest average number of layers of hypodermal cells on a cross-section is 3.1. Resin canals are located medially, and their average number is 5.5. The largest number of sheath cells around the resin canals is 12.8, while the smallest average number is 7.9.

The average tracheid length and width of one-year shoots are 1.219 mm and 26.9 μm respectively. Hybrids *nith* have the widest tracheids of all analysed groups.

F_1 hybrids *thni*
F_1 hibridi *thni*

The average needle length is 9.6 cm. The average fascicle sheath length is 1.0 cm. In the middle of the needle length, there are nine ventral and fifteen dorsal stomatal rows. On a 1 cm long segment in the middle of the needle along the ventral stomatal rows, there are 109 stomata. On the same segment, along one needle rim, there are thirty-five serrations. The average area of th needle cross-section is 1.1550 mm², its height is 1.082 mm, and its width 1.520 mm. Of all analysed groups, hybrids *thni* have the largest area and height of the needle cross-section. The stellar region cross-section area is 0.3032 mm²; its height is 0.449 mm, and its diameter is 0.855 mm. The largest average number of layers of hypodermal cells on a cross-section is 3.0. Resin canals are located medially, and their average number is 8.9, which is the largest number of medial resin canals of all analysed species and hybrids. The largest number of sheath cells around the resin canals is 11.7, while the smallest average number is 7.0.

The average tracheid length and width of one-year shoots are 1.366 mm and 26.5 μm respectively.

Table 36 shows correlation coefficients of all species and hybrids, of the traits that are expected to be related.

Table 36. Correlation coeficients
Tablica 36. Koeficijenti korelacije

Traits *Svojstva*	*ni*	*de*	*th*	*nide*	*deni*	*nith*	*thni*
LI - LR	0.60	0.39	0.52	0.67	0.76	0.29	0.76
LT - DT	0.31	0.83	0.36	0.26	0.04	0.53	0.19
NPPU - NPPV	0.67	0.58	0.52	0.63	0.28	0.43	0.49
PPP- HPP	0.81	0.56	0.87	0.82	0.74	0.76	0.80
PPP - DPP	0.93	0.87	0.97	0.95	0.98	0.91	0.94
PPP - PCC	0.93	0.89	0.94	0.95	0.93	0.94	0.87
PPP - HCC	0.84	0.75	0.90	0.87	0.87	0.72	0.58
PPP - DCC	0.90	0.82	0.87	0.92	0.87	0.86	0.87
HPP - DPP	0.65	0.36	0.81	0.69	0.72	0.52	0.66
HPP - PCC	0.75	0.37	0.87	0.78	0.76	0.78	0.82
HPP - HCC	0.67	0.29	0.86	0.73	0.67	0.55	0.55
HPP - DCC	0.73	0.45	0.79	0.75	0.82	0.76	0.79
DPP - PCC	0.88	0.71	0.92	0.93	0.90	0.82	0.78
DPP - HCC	0.73	0.49	0.81	0.83	0.82	0.56	0.38
DPP - DCC	0.91	0.84	0.91	0.94	0.87	0.82	0.86
PCC - HCC	0.90	0.89	0.91	0.92	0.95	0.80	0.73
PCC - DCC	0.95	0.81	0.95	0.97	0.88	0.89	0.94
HCC - DCC	0.76	0.52	0.75	0.83	0.72	0.47	0.50

The correlation coefficients of the species and hybrids vary between the trait pairs, needle lengths and fascicle sheath length (*LI - LR*), the one-year shoot tracheid width and length (*LT - DT*), and the number of ventral and dorsal stomatal rows (*NPPU - NPPV*). However, they are not high, and it can be concluded that all traits should be included into the discriminant analysis. Correlation coefficients higher than 0.8 are considered as high. If we look at the correlation between the area, height and width of the needle cross-section and the stellar region cross-section, we find high correlation coefficients of the following pairs of traits: the area and diameter of the needle cross-section (*PPP - DPP*); the areas of the needle cross-section and those of the stellar region (*PPP - PCC*); the area of the needle cross-section and the diameter of the stellar region cross-section (*PPP - DCC*); the diameters of the needle cross-section and those of the stellar region (*DPP - DCC*); the area and the height of the stellar region cross-section (*PCC - HCC*), except for hybrid *thni;* the area and the diameter of the stellar region cross-section (*PCC - DCC*). With no considerable significance of the accuracy of determination, it is in future sufficient to measure only one of the two traits that are mutually highly correlated.

Discriminant analysis
Diskriminacijska analiza

Table 37 shows three traits by which the analysed species and hybrids are best discriminated. This is a list of variables which best contribute to group discrimination. However, by using only these traits, the accuracy of discrimination would be lower than shown in the results of the research, where nineteen different traits were included into the analysis.

Table 37. Three traits by which the species are discriminated, separated by the discriminant analysis from nineteen analysed traits

Tablica 37. Tri svojstva po kojima se grupe najbolje razlikuju, izdvojena diskriminacijskom analizom od devetnaest analiziranih svojstava

Analysis *Analiza*	Groups *Grupe*	1st trait *1. svojstvo*	2nd trait *2. svojstvo*	3rd trait *3. svojstvo*
1	*ni - nide*	*NSKM*	*HCC*	*NHmax*
	ni - deni	*NSKH*	*DT*	*LI*
	de - nide	*PPP*	*NSKM*	*NZ/cm*
	de - deni	*PPP*	*PCC*	*DPP*
	nide - deni	*PPP*	*DCC*	*HCC*
2	*ni - nith*	*DPP*	*DCC*	*HPP*
	ni - thni	*LT*	*LI*	*HCC*
	th - nith	*NZ/cm*	*LT*	*DCC*
	th - thni	*DPP*	*DCC*	*NSKM*
	nith - thni	*DPP*	*PCC*	*NSKM*

First analysis: *ni, de, nide, deni*
Prva analiza: *ni, de, nide, deni*

F_1 hybrids *nide* best differ from the black pine by the number of medial resin canals (*NSKM*), by the height of the stellar region cross-section (*HCC*), and by the highest number of hypoderm layers in the needle cross-section (*NHmax*). The average values of all three traits are higher with black pine than with the hybrids.

In discriminating F_1 hybrid *nide* from the Japanese red pine, the following are the most useful traits: the area of the needle cross-section (*PPP*), the number of medial resin canals (*NSKM*), and the number of serrations along one needle rim on a 1 cm-long segment taken from the middle of the needle (*NZ/cm*). The average values of *PPP* and *NSKM* are higher with hybrids than with the Japanese red pine, while the values of *NZcm* are lower in hybrids and higher in the Japanese red pine.

The following three traits are best for discriminating F_1 hybrids *deni* from the European black pine: the number of external resin canals (*NSKH*); the tracheid width of one-year old shoots (*DT*), and the needle length (*LI*). The average *NSKH* is in hybrids higher than in the black pine, which has medial resin canals. The average values of *DT* and *LI* are higher in the black pine than in hybrid *deni*.

F_1 hybrids *deni* are best discriminated from the Japanese red pine by the following: the area of the needle cross-section (*PPP*); the area of the stellar region cross-section (*PCC*), and the diameter of the needle cross-section (*DPP*). The hybrids have higher average values of all three traits than the Japanese red pine. Next trait in discrimination significance is the number of medial resin canals (*NSKM*). The average *NSKM* is higher than in hybrids, since the Japanese red pine has resin canals located near the hypoderm.

The following are the most important characteristics for distinguishing F_1 hybrids *nide* and *deni*: the area of the needle cross-section (*PPP*); the diameter of the stellar region cross-section (*DCC*), and the height of the stellar region cross section (*HCC*). The average value of *PPP* is higher in hybrids *nide*, while the average values of *DCC* and *HCC* are higher in hybrids *deni*. The fourth trait in discriminating the hybrids is the sheath fascicle around the needle (*LR*), which is averagely longer in *nide* than in *deni*.

Second analysis: *ni, th, nith, thni*
Druga analiza: *ni, th, nith, thni*

F_1 hybrids *nith* best differ from the European black pine by the diameter of the needle cross-section (*DPP*), the diameter of the stellar-region cross-section (*DCC*), and the height of the needle cross-section (*HPP*). The average values of *DPP* and *DCC* are higher in the European black pine, while the average *HPP* is higher in hybrids *nith* (Table 70). The next trait for distinguishing these groups is the area of the needle cross-section (*PPP*), which is larger than in the European black pine. Since this is in correlation with the previous traits, we

shall mention the fifth, needle length (*LI*), which is averagely greater than in the European black pine than in hybrids *nith* .

In discriminating F_1 hybrid *nith* from the second parent, the Japanese black pine, the following are the most useful traits: the number of serrations along one needle rim on a 1 cm-long segment taken from the middle of the needle (*NZ/cm*); tracheid length of one-year old shoots (*LT*), and the diameter of the stellar region cross-section (*DCC*). The Japanese red pine has averagely higher values of *NZ/cm* and *LT*, while hybrids have higher values of *DCC*.

The following three traits are best for discriminating F_1 hybrids *thni* from the European black pine: tracheid length of one-year old shoots (*LT*), needle length (*LI*), and the height of the stellar region cross-section (*HCC*). Hybrids *thni* have higher average values of *LT* and *HCC*, while the European black pine has higher *LI* values.

Of all analysed traits, the samples of hybrid *thni* are best discriminated from the Japanese black pine by the following: the diameter of the needle cross-section (*DPP*); the diameter of the stellar region cross-section (*DCC*), and the number of medial resin canals (*NSKM*). Hybrids *thni* have higher average values of all three traits than the Japanese black pine.

F_1 hybrids *nith* and *thni* are best discriminated from one another by the following: the diameter of the needle cross-section (*DPP*), the area of the stellar region cross-section (*PCC*), and the number of medial resin canals (*NSKM*). Hybrids *thni* have higher average values of all three traits than hybrids *nith*.

Needle volatiles composition
Sastav eteričnih ulja iglica

In terms of quality, needle volatiles of the analysed species and hybrids have similar compositions. However, they differ in terms of quantity. These differences are presented in the research results. Our research data on the composition of the European black pine volatile are comparable with those found in other literature.

The volatile composition of *P. nigra* Arnold ssp. *nigra* from Bosnia and Hercegovina was analysed by Chalcat & Gorunović (1995a). They identified 91 components with 90% of the volatile contents. (In our research, we identified fifty-three components with 94.4% of the volatile contents). Besides the differences in terms of quality, the biggest quantitative difference was the content of germacrene D, which they found in traces (in our research, germacrene D is the second (17.7%) after α-pinene as to the proportion of the volatile oil components. The needle volatile analysed by Chalachat & Gorunović (1995a) had a larger proportion of α-pinene (66.5%) and β-phellandrene + limonene (6%), and a smaller proportion of β-pinene (5.3%) than the values in our research (α-pinene (42.7%), β-phellandrene + limonene (3.7%), β-pinene (11.6%)).

The data on the content of terpenes in the European black pine, in terms of quantity, are very similar to the results of the research done by Kubeczka & Schultze (1987). The proportion of germacrene D is 18.6% (17.7% in our research), and this is also the second component after α-pinene, as to the proportion in the volatile. There are quality differences,

since Kubeczka & Schultze (1987) identified only 25 components.

We made a comparison of the needle volatile contents in F_1 hybrids of the first and the second analysis. The components whose proportions in all analysed F_1 hybrids (*nide, deni, nith* and *thni*) are intermediary between the respective components in the parent species are as follows: α-pinene, β-bourbonene, β-caryophyllene, germacrene D and α-muurolene (Tables 34 and 35). These components would be suitable for the identification of the given hybrid combinations.

CONCLUSIONS
ZAKLJUČCI

1. Based on the nineteen analysed traits, it is possible, with a probability of 95% - 100%, to distinguish the hybrids *P. nigra* × *P. densiflora, P. densiflora* × *P. nigra,* and *P. thunbergiana* × *P. nigra* from their parent species. The hybrids *P. nigra* × *P. thunbergiana* are significantly different from the male parent, the Japanese black pine, but not from the female parent, the European black pine.

2. F_1 hybrids *P. nigra* × *P. densiflora* are best discriminated from the European black pine by the number of medial resin canals, the height of the stellar region cross-section, and by the largest number of hypoderm layers on the needle cross-section. The best traits for discriminating these hybrids from the Japanese red pine are the area of the needle cross-section, the number of medial resin canals and the number of serrations along one needle rim on a 1 cm large segment taken from the middle of the needle.

3. The characteristics by which F_1 hybrids *P. densiflora* × *P. nigra* are best discriminated from European black pine are the number of external resin canals, tracheid lengths of one-year old shoots, and needle length. The same hybrids are best distinguished from the Japanese red pine by the area of the needle cross-section, the area of the stellar region cross-section, and the diameter of the needle cross-section.

4. Hybrids *P. nigra* × *P. thunbergiana* are best discriminated from the European black pine by the diameters of both the needle cross-sections and the stellar regions, and the heights of the needle cross-section. Their discrimination from the Japanese black pine is best by the following: the number of serrations along one rim on a 1 cm large segment from the middle of the needle; the tracheid length of one-year old shoots, and by the diameter of the stellar region cross-section.

5. The characteristics by which hybrids *P. thunbergiana* × *P. nigra* are best discriminated from the European black pine are tracheid length of one-year old shoots, needle length and the height of the stellar region cross-section. These hybrids are best distinguished from the Japanese black pine by the diameters of the needle cross-sections, the diameters of the stellar region, and the number of medial resin canals.

6. The most important characteristics for the discrimination of hybrids *P. nigra* × *P. densiflora* and *P. densiflora* × *P. nigra* are the areas of the needle cross-sections, diameters and heights of the stellar region cross-sections.

7. The discrimination of F_1 hybrids *P. nigra* × *P. thunbergiana* and *P. thunbergiana* × *P.*

nigra is best by the diameters of the needle cross-sections, the areas of the stellar region cross sections, and the number of medial resin canals.

8. The volatile compositions of three pine species and their four hybrids, in terms of quality, are very similar, which means that there are small differences in the identified components. However, there are significant differences in terms of quantity, that is the proportions of the individual components are different in different species and hybrids.

9. In the volatiles of the analysed species and hybrids, different numbers of components were detected, ranging from 78 (*P. thunbergiana* × *P. nigra*) to 124 (*P. nigra* × *P. densiflora*). Of the detected components, forty-two were identified in *P. thunbergiana* × *P. nigra*, and fifty-three in the majority of other groups. Of the total content of volatiles, the identified components range between 81.3% (*P. densiflora*) and 96.1% (*P. thunbergiana* × *P. nigra*).

10. The identified components are terpenes, except for *trans*-2-hexenal, which is an n-alkene. Monoterpenes have the largest proportions (between 61.7% in *P. densiflora* and 77.3 % in *P. thunbergiana*), and are followed by sesquiterpenes (from 13.6% in *P. densiflora* to 27.1% in *P. nigra*) and diterpenes (from 0.6% in *P. nigra* × *P. thunbergiana* to 5.9% in *P. densiflora*).

11. Of all the components of the needle volatiles, in all analysed species and hybrids, α-pinene has the largest proportions, ranging between 25.8% in *P. densiflora* to 42.7% in *P. nigra*, except for the Japanese black pine, where β-pinene has the largest proportion (34.1%).

12. Every species has some volatile components with much higher proportions than in the volatiles of other analysed species. The component specific of the European black pine is germacrene D; thunbergol is specific of the Japanese red pine, while β-pinene has the largest proportion in the Japanese black pine.

13. In F_1 hybrids, the proportion of single components is higher, smaller or mostly intermediary when compared to the proportions of the respective components in the volatile of the parent species. The components, whose proportions in all analysed F_1 hybrids are intermediary between the respective proportions of the parent species are α-pinene, β-bourbonene, β-caryophyllene, germacrene D and α-muurolene. Bornyl acetate is a component, whose proportion in all F_1 hybrids is larger than in the parent species, while the proportions of 13-epimanoyl oxide are smaller than in the parent species. We can assume that the mentioned components could be used in the verification of hybrid plants.

14. Cluster analysis has shown that the Japanese black pine and its hybrids (*P. nigra* × *P. thunbergiana* and *P. thunbergiana* × *P. nigra*) differ to a higher degree from other species and hybrids. Another unit is composed of two groups. The first includes the European black pine and its hybrids, where the European black pine is the female parent, *P. nigra* × *P. densiflora*. The second group consists of the Japanese red pine and the hybrids to whom the Japanese red pine is the female parent, *P. densiflora* × *P. nigra*.

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MORFOMETRIJSKA ANALIZA I SASTAV ETERIČNIH ULJA IGLICA NEKIH VRSTA BOROVA I NJIHOVIH HIBRIDA

SAŽETAK

Četiri vrste borova, europski crni bor (*Pinus nigra* J. F. Arnold), obični bor (*P. sylvestris* L.), japanski crveni bor (*P. densiflora* Siebold et Zucc.) i japanski crni bor (*P. thunbergiana* Franco), upotrijebljene su u razdoblju od 1958. do 1991. godine u Zavodu za šumarsku genetiku i dendrologiju Šumarskoga fakulteta Sveučilišta u Zagrebu za proizvodnju međuvrsnih hibrida F_1 generacije, F_2 generacije, povratnih hibrida i trispecies hibrida. Proizvodnja i vrednovanje tih biljaka dugotrajan je proces. Proizvodnja je zahtijevala kontroliranu hibridizaciju na stablima, dvogodišnji razvoj češera, sjetvu sjemena i uzgoj biljaka u rasadniku, te podizanje pokusnih ploha. Proizveden je velik broj hibridnih biljaka, koje su zasađene na četrnaest pokusnih ploha na području Đurđevačkih pesaka i u Arboretumu Lisičine. Od osnutka pokusnih ploha kontinuirano se prati uspijevanje i rast hibridnih biljaka u odnosu na kontrolne biljke čistih vrsta i u odnosu na druge hibridne kombinacije, a rađena su i različita morfometrijska istraživanja.

Ovaj je rad doprinos vrednovanju kontrolirano proizvedenih hibridnih biljaka s obzirom na sličnost koju pojedine hibridne kombinacije pokazuju sa svojim ishodišnim vrstama. Opisan je veći broj morfoloških i anatomskih karakteristika iglica i izbojaka triju vrsta borova i njihove četiri hibridne kombinacije. Također je rađena analiza sastava eteričnih ulja iz iglica tih vrsta i hibrida.

Uzorci su za analizu bili jednogodišnji, potpuno razvijeni izbojci s iglicama, ubrani krajem listopada 1996. godine. Stabla borova s kojih su uzimani uzorci nalaze se na pokusnim plohama u Đurđevačkim peskima (četiri plohe) i u Arboretumu Lisičine (pet ploha), a matična se stabla nalaze na Šumarskom fakultetu u Zagrebu. Sa svakoga su stabla ubrana dva jednogodišnja izbojka. Uzorci su uzeti za tri vrste borova (*P. nigra*, *P. densiflora* i *P. thunbergiana*) i četiri kombinacije križanja tih vrsta (*P. nigra* × *P. densiflora*, *P. densiflora* × *P. nigra*, *P. nigra* × *P. thunbergiana* i *P. thunbergiana* × *P. nigra*).

Jednogodišnji izbojci i iglice rabljeni su za morfološku i anatomsku analizu. Iz svježih iglica svake od vrsta, odnosno hibrida destilacijom vodenom parom dobivena su eterična ulja koja su upotrijebljena za daljnju analizu.

Analizirana morfološka i anatomska obilježja su duljina iglica, duljina rukavca, duljina i širina traheida jednogodišnjih izbojaka, broj pruga puči s unutrašnje i s vanjske strane iglice, tpo promjeru poprečnoga presjeka iglice, površini poprečnoga presjeka centralnoga cilindra i po broju medijalno smještenih smolnih kanala.

Kemijskim analitičkim metodama (plinskom kromatografijom i plinskom kromatografijom/spektrometrijom masa) kvalitativno i kvantitativno određen je sastav eteričnih ulja iglica navedenih vrsta i hibrida.

Detektiran je različit broj komponenti (od 78 za *P. thunbergiana* × *P. nigra* do 124 za *P. nigra* × *P. densiflora*). Od detektiranih komponenti identificirano je od 42 komponente za *P.*

thunbergiana × *P. nigra* do 53 za većinu ostalih grupa. Od ukupnoga sadržaja eteričnoga ulja identificirane komponente čine od 81,3 % za *P. densiflora* do 96,1 % za *P. thunbergiana* × *P. nigra*.

Identificirane su komponente terpeni, osim *trans*-2-heksenala koji je n-alken. Najveći je udio monoterpena (61,7 % kod *P. densiflora* do 77,3 % kod *P. thunbergiana*), zatim slijede seskviterpeni (13,6 % kod *P. densiflora* do 27,1 % kod *P. nigra*) i diterpeni (0,6 % kod *P. nigra* × *P. thunbergiana* do 5,9 % kod *P. densiflora*).

Od komponenti prisutnih u eteričnom ulju iglica kod svih je analiziranih vrsta i hibrida najveći udio α-pinena (25,8 % kod *P. densiflora* do 42,7 % kod *P. nigra*), osim kod japanskoga crnoga bora kod kojega je najveći udio β-pinena (34,1 %).

Za svaku vrstu postoje komponente koje u eteričnom ulju dolaze u višestruko većem udjelu nego u eteričnom ulju ostalih analiziranih vrsta. Komponenta specifična za europski crni bor je germakren D, za japanski crveni bor tunbergol, a za japanski crni bor β-pinen.

Kod F_1 hibrida udio pojedinih komponenti je veći, manji ili najčešće intermedijaran u odnosu na udio istih komponenti u eteričnim uljima roditeljskih vrsta. Komponente čiji je udio kod svih analiziranih F_1 hibrida intermedijaran u odnosu na udio kod roditeljskih vrsta su α-pinen, β-bourbonen, β-kariofilen, germakren D i α-murolen. Bornil-acetat je komponenta čiji je udio kod svih F_1 hibrida veći nego kod roditeljskih vrsta, a 13-epimanoil-oksida ima kod svih hibrida manje nego kod roditeljskih vrsta. Navedene bi se komponente mogle rabiti za verifikaciju hibridnih biljaka.

Cluster analiza je pokazala da su po sastavu eteričnih ulja iglica japanski crni bor i njegovi hibridi (*P. nigra* × *P. thunbergiana* i *P. thunbergiana* × *P. nigra*) znatnije različiti od ostalih vrsta i hibrida. Druga je cjelina sastavljena od dviju grupa. Prvu grupu čini europski crni bor i hibridi kojima je on ženski roditelj *P. nigra* × *P. densiflora*. U drugoj su grupi japanski crveni bor i hibridi kojima je ova vrsta ženski roditelj, *P. densiflora* × *P. nigra*.

Ključne riječi: *Pinus nigra* J. F. Arnold, *P. densiflora* Siebold et Zucc., *P. thunbergiana* Franco, međuvrsni hibridi, morfologija iglica, anatomija iglica, traheide izbojaka, diskriminacijska analiza, eterična ulja, GC, GC/MS, terpeni, *cluster* analiza