COMPARATIVE STUDY OF THE SECRETORY STRUCTURES AND VOLATILE OILS IN SOME PINUS SPECIES

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Abstract: The research is focused on the comparative analysis of volatile oils extracted through hidrodistillation from the leaves of 4 Pinus species. The differences between the secretory structures (resin canals) were underlined. The volatile oils were analyzed through GC-FT-IR, a complementary method of the mass spectrometry, which allowed a qualitative analysis comparing the obtained specters with that of the library. The analysis put in evidence over 20 compounds characteristic for the volatile oils in general (\(\alpha\) and \(\beta\)-pinene, terpineol, linalool, bornil acetate, ocimen, etc). The volatile oils of the Pinus species have a different composition correlate with the species, phenophases, chemotype.

Key words: resin ducts, volatile oils, Pinus

Introduction

In conifers, the resin duct is a common structure of the plant body. It may be present both in the primary and secondary tissues; but it seems to occur more consistently in leaves. Previous research has examined the structure, distribution, and development of resin ducts in the Pinaceae [3, 5, 7].

Napp-Zinn (1966) classified four types of resin ducts according to their position in the needles of Pinus: (1) ducts in contact with the hypodermis (i.e., external); (2) ducts surrounded by chlorenchyma (i.e., medial); (3) ducts in contact with the bundle sheath (i.e., endodermis) in the chlorenchyma (i.e., endonal); and (4) ducts inside the bundle sheath.

The resin duct is an important character, applied in classifying the Pinaceae and particularly in distinguishing Pinus species [1].

The number and position of resin ducts in needles may vary considerably and interspecifically in pines [6]. Nevertheless, for the identification of pine species, the number of resin canals in the needles is of no particular importance, except for a few species that normally only have two or three canals; however, the relative position of the ducts in the needle may be used as an aid in identification.

Material and methods

Biochemical study – The volatile oils was extracted from the needles was separated by water-steam distillation (Neo-Clevenger- distillation apparatus) were analyzed by gaschromatograph and mass spectrometry. The separation of the components was made in a gaschromatograph (using a capillary column DB5 / 25m, diameter – 0,25 mm). The initial temperature was 60 °C and was elevated with 5°C/minute until 280 °C.

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For the identification of the compounds of volatile oils the chromatograph was linked with a Fourier transform infrared spectrometer (FRIR) Nicolete. The quantitative analysis was made with a flame-ionizing detector. For FRIR spectrometer a spectral domain of 4000 - 750 cm\(^{-1}\) was used (8 cm\(^{-1}\) resolution and an acquisition speed of 7 scan/s). In the same time, the volatile oil was investigated with a gaschromatograph (with massspectrometric detector) with an ionic trap, Varian Saturn II linked with a chromatograph (3400). The Kovats retention indices were used for confirmation of the exact position of the peaks on the chromatogram using the n-alkanes as internal standards.

**Histo-anatomical methods** – The vegetal material (needles of *Pinus sylvestris*, *P. ponderosa*, *P. strobus*, *P. nigra*) were collected from Iassy Botanical Garden and Bucharest Botanical Garden, fixed and conserved in ethanol 70%. The sections (at three compatible levels – base, middle and top) were made with free hand using a razor blade and colored with red-ruthen and methyl-blue. The photos were made after the obtained permanent slides using a Novex (Holland) microscope and a Minolta photo camera.

**Results and discussions**

The *biochemical analysis* of the volatile oils extracted from *Pinus strobus* (Fig. 1) needles evidenced 9 major compounds (Fig. 1) (44% α-pinene, 26 % β-pinene, 8% terpinolene, 8% camphene). In small quantities (< 4%) myrcene, β - caryophyllene and α-terpinene was identified.

From the volatile oil extracted from *Pinus sylvestris* (Fig. 2) needles 11 principal compounds was identified (the major compound was α-pinene – 83%). Another compounds were observed in small quantities (< 4%). Some of them were commune in the volatile oil extracted from *Pinus strobus* (β-pinene, myrcene, β – caryophyllene), but some one are different (like ocimene, bornyl acetate, terpinyl acetate, hidrocarvyl acetate and farnesol.

The composition of the volatile oil from *Pinus ponderosa* (Fig. 3) has important similarities with that extracted from *Pinus strobus*. The major compounds were α-pinene (22%), β-pinene (50 %), β-caryophyllene (12%). Some specifically compounds were identified: camphor, farnesol, linalool, bornyl acetate, carvyl acetate and ethyl laurate.

In the volatile oil extracted from *Pinus nigra* needles (Fig. 4) 14 major compounds was identified: α-pinene (68%), β – caryophyllene (12 %). This oil present some similarities with that extracted from *P. sylvestris*. In difference, some specific compounds were identified: terpinen - 4 – ol, carvyl acetate, verbenone and camphor.

If we compare the obtained data we will notice that α-pinene, β-pinene and β – caryophyllene are commune for all 4 investigated species. For each species some specific compounds were identified (Fig. 5).

**Anatomical results:**

*Pinus sylvestris* – the majority of the secretory canals are in contact with the hypodermis or surrounded by chlorenchyma; each canal is completely or partial surrounded by schlerenchyma fibres, with thick and lignified walls. Sometime, ducts inside the bundle sheath could be observed. The number of ducts is variable in the length of the needle (2-4 at the basis, 10-12 in the middle and 2-3 in the top).
Pinus nigra – the secretory canals are located into the chlorenchyma; in all analyzed samples were no ducts in contact with the hypodermis. The number of the ducts is relative constant in the length of the needle (9 at the basis, 12-13 at the middle, 11-12 at the top).

In Pinus ponderosa needles all secretory canals are in medial positions (surrounded by chlorenchyma). At the basis two secretory ducts could be observed; they are large, surrounded by 1 or 2 layers of mechanic cells with relatively thin walls. In the middle of the needles 3 (at a young leaf) or 4-5 (at a mature leaf) ducts, all in the assimilatory tissue, are visible. In the top of the needle are 2 secretory canals in the same position.

In Pinus strobus only one or two ducts could be observed in the length of the needle. They are in contact with the hypodermis, someone in direct contact with epidermis.

Conclusions

The biochemical investigations reveal high concentrations of $\alpha$-pinene, $\beta$-pinene and $\beta$ – caryophyllene in all analyzed species; $\alpha$ – pinene have more than 50% in Pinus sylvestris and Pinus nigra, Pinus ponderosa has important similarities with that extracted from Pinus strobus.

The number and distribution of secretory canals are different in all 4 analyzed species. In Pinus sylvestris and Pinus nigra needles they are numerousness, distributed around the central cylinder (more in external position in Pinus sylvestris, and in medial position in Pinus nigra); ducts inside the bundle sheath could be observed only in Pinus sylvestris. In Pinus ponderosa and Pinus strobus the number of the ducts is smaller; they are in medial (Pinus ponderosa) or in external (Pinus strobus) positions. The histo-anatomical investigations confirm the biochemical similarities between Pinus sylvestris and Pinus nigra, Pinus ponderosa and Pinus strobus.

BIBLIOGRAPHY


Explanations of photos:
1 – Crosssection from the P. sylvestris needle (x100); 2 - Crosssection from the P. sylvestris needle (x400); 3 - Crosssection from the P. nigra needle (x200); 4 - Crosssection from the P. ponderosa needle (basis) (x200); 5 - Crosssection from the P. ponderosa needle (middle) (x200); 6 - Crosssection from the P. strobus needle (x200)
Fig. 1 – The composition of volatile oil from *Pinus strobus* (up – the percent concentration, down – the position of the peaks on the chromatogram)
Fig. 2 – The composition of volatile oil from *Pinus sylvestris* (up – the percent concentration, down – the position of the peaks on the chromatogram)
Fig. 3 – The composition of volatile oil from *Pinus ponderosa* (up – the percent concentration, down – the position of the peaks on the chromatogram)
1. Pinus nigra

- \(\alpha\)-pinen
- \(\beta\)-pinen
- \(\gamma\)-pinen
- ocimen
- terpinolen
- camfor
- terpinen-4-ol
- \(\alpha\)-terpeneol
- verbenona
- bornil acetat
- carvil acetat
- terpinil acetat
- beta-cariofilen
- farnesol

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Fig. 4 – The composition of volatile oil from Pinus nigra (up – the percent concentration, down – the position of the peaks on the chromatogram)