Scientific Papers, USAMV Bucharest, Series A, Vol. LII, 2009, ISSN 1222-5339

THE STRUCTURE OF THE SECRETIVE TISSUES AND CONTENT OF THE VOLATILE OIL IN SOME SPECIES OF GYMNOSPERMS

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Keywords: *Pinus, Thuja, volatile oil, microscopic researches*

Abstract

Research has mentioned that Pinus sylvestris L. specie has more than 5 secretive channels. Abies alba Miller has two secretive channels, and Thuja orientalis L. has a single secretive channel.

The analysis of the volatile oil extracted from the leaves of these species revealed the presence, as priority compounds, of the following substances: α-pinen in volatile oil of Pinus sylvestris, bornile acetate, β-pinen, camphene and α-pinen, in that from Abies alba and α-pinen in the cold season and thujana, during summer, in volatile oil from Thuja orientalis.

INTRODUCTION

Secretive tissues in gymnosperm plants are mainly represented by the secretive channels [4, 9]. Toma a.o. (2003) carried out a comparative survey on secretive tissues, in eight species of *Pinus*, and further, Toma and Rugina (1998) studied the secretive channels in *Juniperus communis L.* Ivanescu a.o. (2007) investigated the histo-anatomical structure of some species of *Cupressaceae* and observed the presence of secretive channels in all studied species, with varied position, number and length.

Research carried out by Wales a.o. (1973) regarding the structure of secretive channels, observed that they are made up of a secretive epithelium, that in the middle delimitates an area where collected substances are gathered and it is covered at the outside by sclerenchimatic tissue.

Observations made by Charon a.o. (1987) shown that leucoplasts in the secretive cells in *Pinus* are surrounded by canalicles of the endoplasmic reticule, and during secretion period, the volume of leucoplasts and their surface increase very much.
The composition of the volatile oil extracted from the leaves of gymnosperm plants was analyzed by number of researchers: Latish a.o. (1984), Chalcat a.o. (1985), Carmo and Frazao (1986), Kubeczca and Schultze (1987), Burzo a.o. (2005), Kurose a.o. (2006), etc.

MATERIAL AND METHODS

Research was carried out on the following species of conifers: *Pinus sylvestris L.*, *Abies alba Miller*, and *Thuja orientalis L.*, originated from Bucharest USAMV Botanic Garden.

For histological analysis, the leaves of conifers were prefixed into a solution of glutaric aldehide in 2.7% concentration, in 0.1M phosphate tampon, pH 7.2 and dehydration for 30 minutes, into acetone solutions under increasing concentration of 50, 60, 70, 80 and 90%. In the end, the samples were included in Epon 812 epoxydic resin, they were semi fine sectioned under an ultra microtone Leica UC6 type, colored in toluidine blue and examined and digitally recorded with an optical microscope Olympus BX 51 type, provided with video camera.

Volatile oils were extracted from the conifer leaves by hydrodistillation, using a Cleverger type device in this purpose.

Separation of compounds was made by a chromatophore in AGILENT gaseous state, provided with a mass spectrometric detector, with quadrupole. A DB 5 type capillary route with a 25 m length and 0.25 mm diameter, using helium as driving gas, and the initial temperature in the oven was 50°C. 4 minutes isotherm and it increased up to 280°C, with a 4°C/minute gradient.

There were also used Kovats retention indices for confirmation of the exact position of the picks in chromatogram.

RESULTS AND DISCUSSION

Microscopic researches showed that in case of *Pinus silvestris L.* specie, in the foliar mesofile located around the dial, the presence of 5-12 secretive channels is observed, disposed on both sides of the leaf, close to the hypodermis. There can be seen in figure 1 that their lumen is surrounded by approximately 9 secretive cells, rich in cytoplasm.

The leaves of *Abies alba* specie have at the two side ends of the leaves, towards the abaxial side, immediately under the hypodermis, one secretive channel. This one has a big lumen, which is surrounded by approximately 18 secretive cells of smaller sizes (figure 2).

On the abaxial side of the leaves of *Thuja orientalis* a secretive channel, with small sizes and characteristic structure is found. Secretive cells, rich in cytoplasm, delimitate the channel lumen. These cells are covered on the outside by a layer of sclerenchimatic cells, flattened, with thickened walls (figure 3).

Quantity analysis of the volatile oil revealed the existence of a variation depending on the season and specie. The largest quantity of volatile oil was determined in summer, when biosynthetic activity is more intense. The quantity of volatile oil extracted from leaves varied depending on the same factors between 0.30 and 0.40

ml/100g of leaves, in *Pinus sylvestris*, between 0.24 and 0.20 ml/100g in *Abies alba*, between 0.09 and 0.25 ml/100g in *Thuja orientalis*.

The analysis of the volatile oil extracted from leaves of *Pinus sylvestris* allowed the identification of 48 compounds, out of which the majority was held by α-pinen, 76.5-80.1%, during spring and summer and by β-pinen (35.8%), in autumn.

The volatile oil extracted from *Abies alba* specie contained a number of 48 compounds, out of which the majority was held by bornile acetate (18.5-24.4%), camphene (16.8-19.6%), β-pinen (15.7-19.0%) and α-pinen (10.0-14.0%).
The highest number of compounds: 63, was determined in the volatile oil extracted from the leaves of *Thuja orientalis* (figure 4).

The main compound in the volatile oil was represented by α-pinen, which during the winter held 53.05% of the total compounds, along with the accumulation of thujone (56.27%).

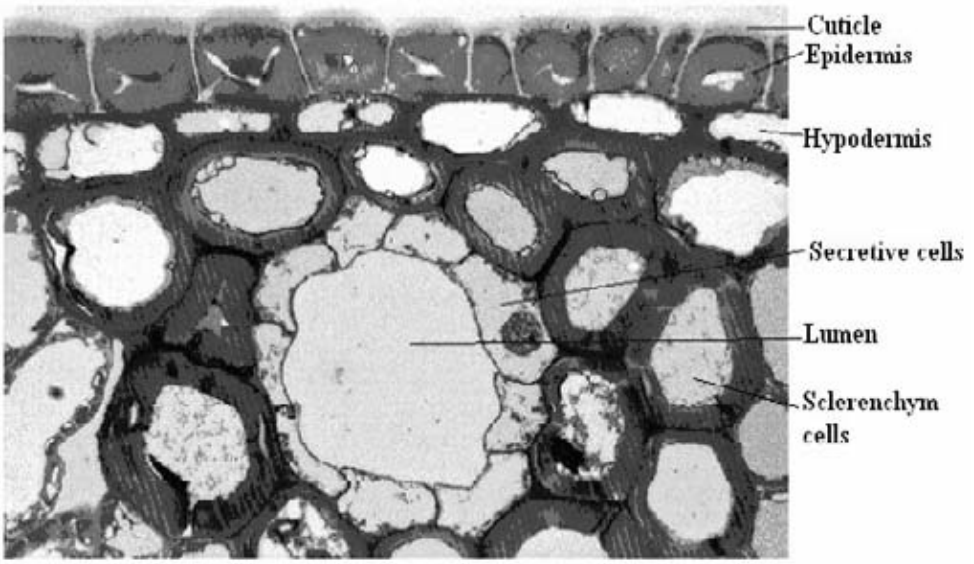

Fig.1 Secretive channel of Pinus sylvestris leaf

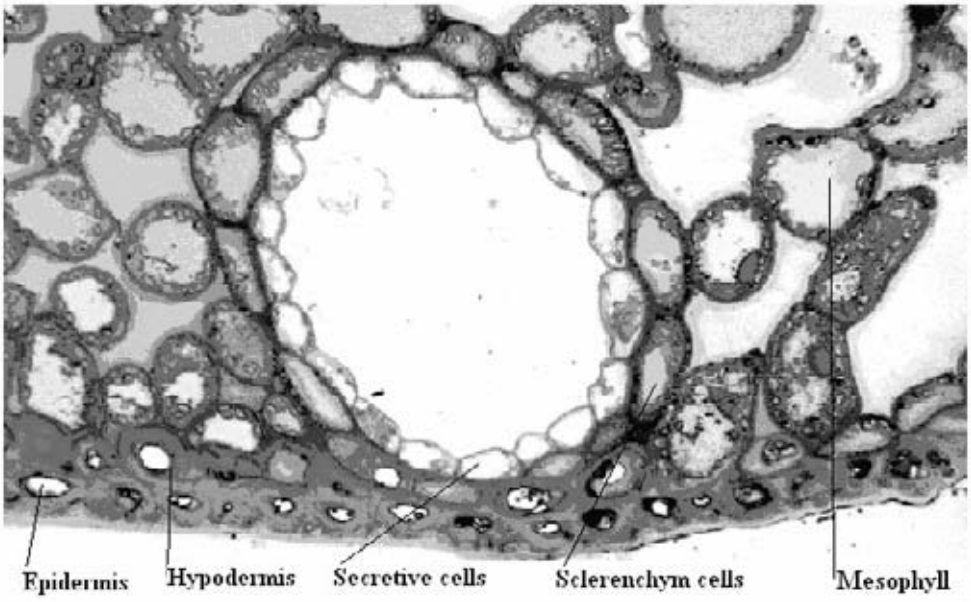

Fig.2 Secretive channel of Abies alba leaf

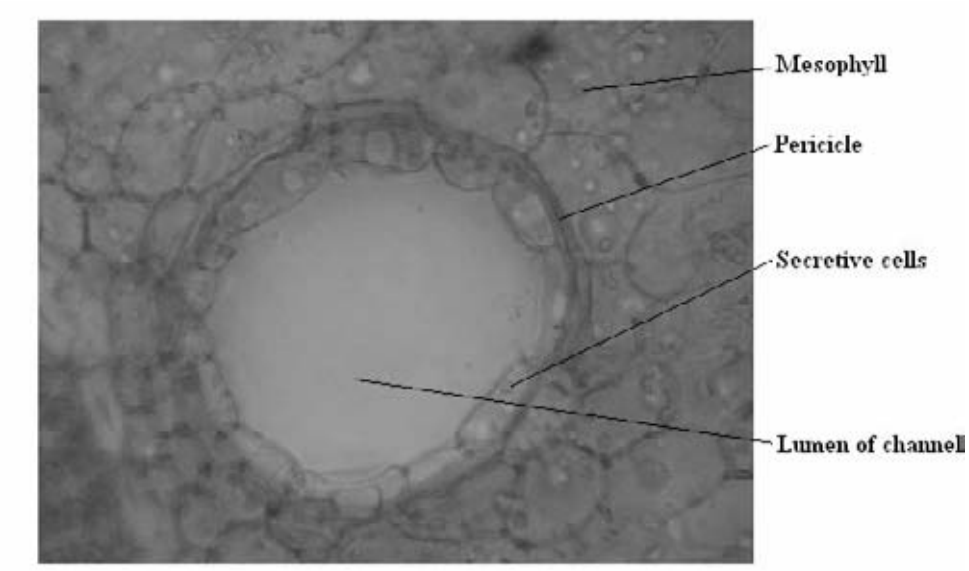

Fig. 3 Transversal section through secretive channel of Thuja orientalis leaves

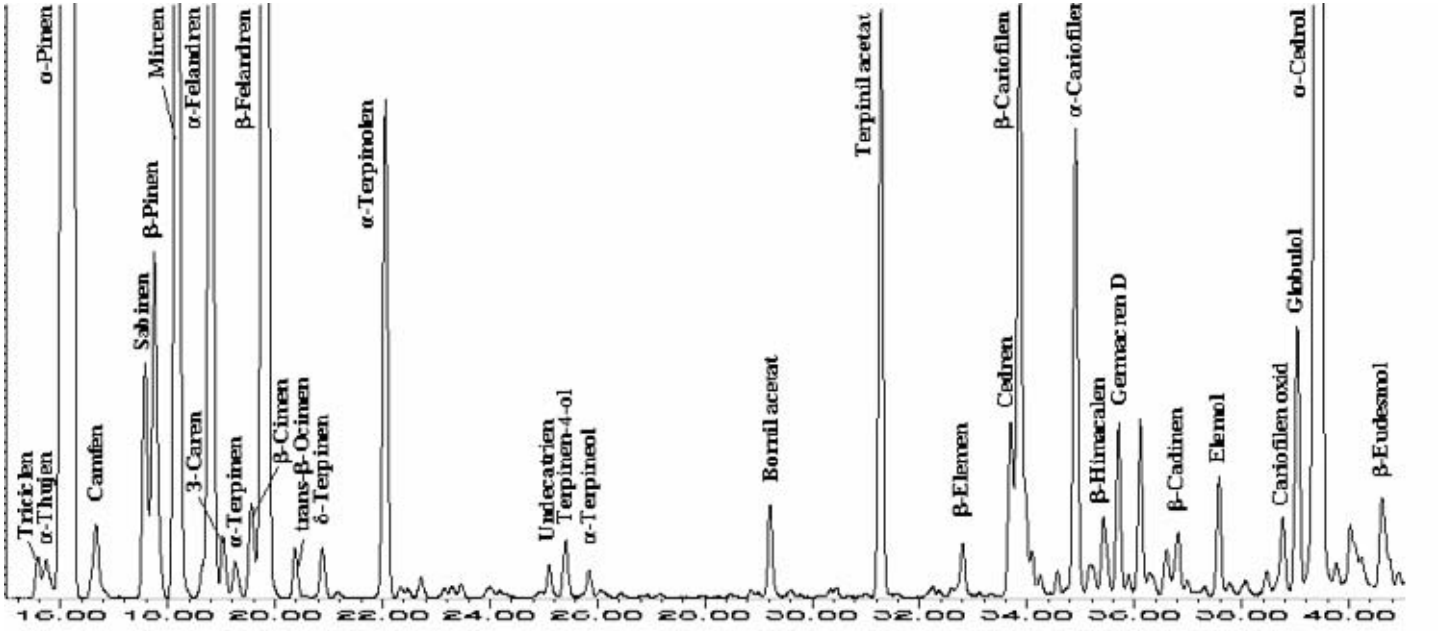

Fig. 4 Gas cromatogram of thuja orientalis

CONCLUSIONS

1. Research carried out showed that *Pinus sylvestris* has more that 5 secretive channels, *Abies alba Miller* has two secretive channels, and *Thuja orientalis L.* has a single secretive channel.
2. The quantity of volatile oil extracted from leaves varied depending on the same factors between 0.30 and 0.40 ml/100 g of leaves, in *Pinus sylvestris*, between 0.24 and 0.20 ml/100 g in *Abies alba*, between 0.09 and 0.25 ml/100 g in *Thuja orientalis.*
3. In *Thuja orientalis* the number identified in the volatile oil was 63, and the individual share varied depending on the season.
4. Priority compounds in volatile oil in *Pinus sylvestris* were represented by α-pinen in *Abies alba* by bornile acetate, β-pinen, camphene and α-pinen, and in *Thuja orientalis* by α-pinen in the cold season and thujone, during summer.

REFERENCES

1. Burzo I., A. Dobrescu, L. Bădulescu, D. Mihaescu, D. Bălan, 2005. *Fiziologia plantelor. Substanţele utile din plante.* Ed. Elisavaros, Bucureşti.
2. Chalchat J.C., R.P. Garry, A. Michet, P. Remery, 1985. *The Essential Oil of Two Chemotyps of Pinus sylvestris (oil of type A).* Phytochemistry. Vol. 24 (pp. 2443-2444).
3. Charon J., J. Launay, J.P. Carde, 1987. *Spatial Organisation and Volume Density of Leucoplasts in Pine Secretory Cells*. Protoplasma, nr. 138 (pp. 45-53).
4. Esau K., 1965. *Plant anatomy*. Ed. 2, John Wiley and Sons edit., Inc. New York.
5. Ivănescu L., C. Toma, R. Rugină, 2007. *Histo-anatomical research regarding some species of Cupressaceae*. Analele Ştiinţifice ale Universităţii Al. I. Cuza Iaşi, Secţiunea II-a, Biologie vegetală, tomul LIII (pp. 1-10).
6. Kubeaczka K., W. Schultze, 2006. *Biology and Chemistry of Conifer Oil*. Flavour and Fragrance Journal, vol. 2, nr. 4 (pp. 137-148).
7. Kurose K., D. Okamura, M. Yatagai, 2006. *Composition of the essential oils from the leaves of nine Pinus species and the cone of three of Pinus species*. Flavour and Fragrance Journal, vol. 22, nr. 1 (pp. 10-20).
8. Latish V.G., R.D. Kolesnikova, and R.I. Derjuzhkin, 1984. *Essential Oils of Some Pine Species*. Proceedings of IXth International Congress of Essential Oils, Technical Paper Book 2, Singapore (pp. 83-89).
9. Napp-Zinn K., 1966. *Anatomie des blattes*. Blattanatomie der Gymnospermen. Gebruder Borntraeger, Berlin.
10. Toma C., R. Rugină 1998. *Anatomia plantelor medicinale*. Ed. Academiei Române, Bucureşti.