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

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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-pinene in volatile oil of Pinus sylvestris, bornyl acetate, β-pinene, camphene and α-pinene, in that from Abies alba and α-pinene in the cold season and thujone, 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 aldehyde 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-pinene, 76.5-80.1%, during spring and summer and by β-pinene (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 bornyl acetate (18.5-24.4%), camphene (16.8-19.6%), β-pinene (15.7-19.0%) and α-pinene (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-pinene, 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](image-url)
Fig. 2 Secretive channel of Abies alba leaf

Fig. 3 Transversal section through secretive channel of Thuja orientalis leaves
Fig. 4 Gas chromatogram of thuja orientalis
CONCLUSIONS

1. Research carried out showed that *Pinus sylvestris* has more than 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-pinene in *Abies alba* by bornyl acetate, β-pinene, camphene and α-pinene, and in *Thuja orientalis* by α-pinene in the cold season and thujone, during summer.

REFERENCES