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TERPENE COMPOSITION OF *PINUS PINASTER* SEEDLINGS AND PLANTS

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Key Word Index—*Pinus pinaster*; Pinaceae; essential oils; α- and β-pinene; variation with growth; genetics.

Abstract—The production of essential oils in *Pinus pinaster* increases with the age of the seedlings, being higher in plants grown under continuous illumination. In the seedlings, nearly all the terpene is α- and β-pinene, the relative proportions of which are completely reversed between the 6th and 10th days of growth, regardless of the illumination period. Another reversion takes place after 60–65 days, the proportion found in the adult pine being very constant with α-pinene as the main component. The amount of oil in seedlings from high resin yielding parent trees was 4-fold higher than that in seedlings of normal (wild type) seeds. This finding is interesting because it can be employed for the preselection at the early stage of seedling of seeds to be used in forestry to obtain plantations of a high resin yield.

INTRODUCTION

THE WIDESPREAD occurrence and economic importance of terpenes has stimulated research concerning ways of improving its release and/or collection from pine. The available information indicates that environmental factors (soil, geography) have only little influence on the composition of the pine resin. For example, results obtained in our laboratory on the effect of different mineral nutrients on *Pinus pinaster* plants grown in hydroponic cultures showed that only the quantity of the resin but not its composition is effected.[1] However, Zavarin *et al.* have reported that there are significative variations in the amount of methyl chavicol and total monoterpenes in *Pinus ponderosa* according to the season and needle ages.[2]

This paper reports the results obtained in the study of the time-course of the composition and quantity of essential oil of *Pinus pinaster* seedlings and plants aged from 1 to 3 months. The resin production in seedlings from normal (wild type) seeds and from seeds of pines selected for their high resin yield is also reported. Our results show that it is possible to establish at an early stage in the life of seedling the resin producing ability of the mature plant.

RESULTS

Influence of Age and Illumination period on the Production of Terpenes by Seedlings

The time-course of resin production shows a clear increase in the total amount of essential oils after the germination of the stratified seeds (Table 1, a). The increase is specially marked between 15 and 30 days, being higher in the series grown under continuous illumination. During this period, nearly all the terpene is α- and β-pinene; no minor constituents are present. The proportion of α- and β-pinene is reversed between the 6th and 10th days of growth, regardless of the illumination period (Table 1, b).

[1] FUNES, A., SÁNCHEZ-MEDINA, F. and MAYOR, F. (1973) *Montes* **160**, 307.
[2] ZAVARIN, E., COBB, JR., F. W., BERGOT, J. and BARBER, H. W. (1971) *Phytochemistry* **10**, 3107.

TABLE 1. TERPENE CONTENT AND COMPOSITION OF *Pinus pinaster* SEEDLINGS*
(Influence of growth and illumination period)

Time of growth (days)	Illumination period: Dark	14-hr illumination	Continuous illumination
(a) *Terpene content*†			
0	1·2	1·2	1·2
3	1·2	3·5	0 8
6	1·3	2·0	0·4
10	2·8	3·5	2·9
15	7·8	61·1	91·7
20	36·2	65·8	146·9
25	31·5	62·2	253·3

Terpenes	Time of growth (days): 0	3	6	10	15	20	25
(b) *Terpene composition*‡							
Dark							
α-pinene	84·6	92·0	72·0	46·0	36 5	35·1	40·0
β-pinene	15 3	8·0	28·0	54·0	63·0	58 0	57·8
Sabinene	—	—	—	—	—	1·6	1·0
Δ^3-Carene	—	—	—	—	—	0·3	0·4
Limonene	—	—	—	—	—	1·6	1·2
14-hr illumination							
α-pinene	84·6	80·3	94·0	43·0	35·0	33·5	33·1
β-pinene	15·3	13·2	6·0	57·0	60·0	63·0	63·5
Sabinene	—	—	—	—	2·9	1 4	0·7
Δ^3-Carene					8·8	0 9	1·0
Limonene					1 7	11 0	1 5
Continuous illumination							
α-Pinene	84·6	92·0	81·0	45 4	33·5	35·5	35·7
β-Pinene	15·3	8·0	19·0	54·6	66·5	51 4	55·8
Sabinene	—	—	—	—	0·4	2·1	1·3
Δ^3-Carene	—	—	—	—	—	1·3	1·3
Limonene	—	—	—	—	—	3·7	22·3
β-Phellandrene	—	—	—	—	—	0·9	0·7
Terpinolene	—	—	—	—	—	5·0	3·9

* The seedlings were grown at 20°, humidity 75–85% and pH 5·5.
† The results are expressed in μl/100 g dry wt and are the mean values of four analyses.
‡ The results are expressed in percentage composition and are the mean values of four analyses

Changes in the Total Amount and Composition of the Essential Oils in Plants

As the resin of *Pinus pinaster* adult tree is mainly composed of α-pinene, the composition of the resin in plants grown for 35, 40, 50, 60, 70 and 90 days was determined to detect any further changes in the relative concentrations of α- and β-pinene. The results obtained in seedlings showed a big increase in β-pinene. However, after 60–65 days, the α-pinene content becomes dominant and this remains the major component from then on.

Resin Content in Seedlings and Plants

All the samples from seedlings and plants obtained after growing stratified seeds of trees selected for their high resin production gave very similar results, both in the amount and composition of the oil. The mean values were always four fold higher than those obtained with normal (wild type) seeds (Table 2).

TABLE 2. TERPENE CONTENT IN SEEDLINGS AND PLANTS OF *Pinus pinaster*

(a) *Seedlings**

Sample	Amount of essential oil	α-Pinene (%)	Sabinene (%)	β-Pinene (%)	Limonene (%)	β-Phellandrene (%)
1	282·7	19·4	1·3	76·5	1·1	1·5
2	270·6	21·0	1·5	74·0	1·3	2·0
3	276·4	19·1	1·0	77·4	—	1·3
4	271·5	21·9	1·0	73·7	—	2·0
5	281·6	20·5	1·2	75·6	—	1·5
6	62·2	31·0	0·7	63·5	—	1·0

(b) *Plants*†

Sample	Amount of essential oil	α-Pinene (%)	Sabinene (%)	β-Pinene (%)	Δ^3-Carene (%)	Limonene (%)	β-Phellandrene (%)
1	463·3	29·1	1·8	66·5	0·5	0·7	1·0
2	435·5	28·9	1·8	66·6	0·7	0·7	1·1
3	468·1	28·0	1·2	67·2	0·8	1·0	1·5
4	116·4	40·4	1·5	54·6	0·4	1·6	1·5

* The seedlings were grown during 25 days on 14 hr illumination, 20°, humidity 75–85% and pH 5·5. Samples 1–5: seedlings grown from seeds of trees characterized by a high resin production. Sample 6: seedlings grown from normal (wild type) seeds (mean values of 10 samples).

† The plants were grown during 2 months on 14 hr illumination, 20°, humidity 75–85% and pH 5·5. Samples 1–3: plants grown from seeds of trees characterized by a high resin production. Sample 4: plants grown from normal (wild type) seeds (mean value of 10 samples). The amount of essential oils are expressed in μl/100 g dry wt, and are the average value of four analyses.

DISCUSSION

It is interesting that the seeds already have essential oils in very low amounts. There is an increase in oil in the seedlings grown under longer illumination periods. Comparing the amount of essential oils after 25 days of growth with the values obtained at the start of the experiment, the ratio is about 220 times higher in the case of the seedlings grown under continuous illumination, whereas the values are only about 60 times higher when the period of illumination is of 14 hr per day and 30 times when growth is carried out in a dark chamber. The increase starts about the 10th day of growth, when the first real plant structures of the seedlings begin to appear.[3] After 15 days of growth, the minor compounds are detected. When plants are illuminated for 14 hr per day, the minor components appear before that time.

When considering the double reversion of the α- and β-pinene percentages (the first after 9 days of growth and the second after 60–65 days), it is interesting that the first reversion

[3] PARDOS, J. A. and MARCOS, J. (1963) *Anal. Inst. Forest. Invest. Exper.* **8**, 219.

takes place when the nutritional sources are only endogenous (of cotyledon origin) and that the second reversion occurs when the plants already have their own means to metabolize exogenous nutrients.

In the comparative study using seeds from cones of pines selected as good resin producers, the existence of a clear genetic factor for essential oil synthesis has been corroborated, both in the composition and in the production of resin.[4–8] This finding is interesting because it can be employed for the preselection (at the seedling stage) of seeds to be used in forestry to to obtain plantations of a high resin production.

EXPERIMENTAL

The extraction of the essential oils was carried out by steam-distillation, using a Neo-Clavenger apparatus The extraction method and the GLC procedure have been described in detail.[9]

Seedlings were grown in a highly controlled chamber, the temp being 20°, humidity 75–85%, and pH 5·5. Three series of samples were grown: (1) seedlings grown in the absence of light; (2) seedlings grown with an illumination period of 14 hr; and (3) seedlings grown under continuous illumination. The analysis has been made in stratified seeds before growing and after 3, 6, 10, 15, 20 and 25 days of growth. The culture conditions of the plants (1–3 months) were identical to those of the seedlings, but after 30 days of growth it was necessary to add some nutrients for normal plant development.[9] The normal (wild type) seeds were supplied by the Department of Forestry from Sierra de Cazorla (Jaen). The seeds of trees selected by their high resin production came from Coca (Valladolid) and were kindly supplied by the Forestry Institute.

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