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MONOTERPENE COMPOSITION OF CORTICAL OLEORESIN FROM DIFFERENT CLONES OF *PINUS SYLVESTRIS*

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Key Word Index—*Pinus sylvestris*; Pinaceae; monoterpenes; inheritance of composition.

Abstract—Monoterpene composition of cortical oleoresin was determined in a number of 9-yr-old grafts of Scots pine (*Pinus sylvestris*), growing in a clone trial at two different levels of mineral nutrient supply. Variation between clones was considerable for some of the monoterpene constituents, indicating genetic control. In one of the clones, Δ^3-carene, α-terpinene and terpinolene were totally absent. The monoterpene composition was not significantly affected by the annual application of macro-nutrients.

INTRODUCTION

IN EARLIER studies a high intraspecific variation in monoterpene composition in both cortical and stem-xylem oleoresin of the genus *Pinus* has been reported.[1–4] Further investigations have indicated that the composition may vary greatly even among individual trees belonging to the same species, and that the differences in the content of some monoterpenes are genetically controlled.[4–8]

The present paper reports data on monoterpene composition of cortical oleoresin from a number of 9-yr-old grafts of Scots pine (*P. sylvestris*.) growing in a clone trial at two different levels of mineral nutrient supply.

RESULTS

The major monoterpenes in the trees sampled were α-pinene, β-pinene, Δ^3-carene and myrcene. Variable, but small amounts of terpinolene, β-phellandrene, limonene, camphene, α-terpinene and γ-terpinene occurred in most trees. The amount of each constituent showed considerable tree-to-tree varation within the same clone.[4,6] For a number of monoterpenes the mean relative contents differed significantly between the clones ($P < 0{\cdot}05$). This was specially true of β-pinene, Δ^3-carene and myrcene. The clone D differed markedly from the other three clones, as Δ^3-carene, α-terpinene and terpinolene were

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totally absent. The proportion of α-pinene, on the other hand, was significantly higher in the clone D as compared to that in the other clones. Thus an identification of the four clones was possible from the monoterpene composition.

A certain reservation should be made for the β-pinene figures. They also include a minor unknown component, probably sabinene (from the MS). Of interest was the finding that the monoterpene composition of the oleoresin was not significantly influenced by the mineral nutrient status of the soil. Although further exploratory work is needed, even for elucidating the seasonal and age effects as well as sampling error, the results (Table 1) indicate a possible approach to utilize the monoterpene composition of the oleoresin as a tool in genetical research on pines.

TABLE 1. PERCENTAGE COMPOSITION OF MONOTERPENES IN CORTEX OLEORESIN OF FOUR CLONES OF SCOTS PINE

	Clone A			Clone B			Clone C			Clone D			Average
	f	*nf*	*f* + *nf*	*f*	*nf*	*f* + *nf*	*f*	*nf*	*f* + *nf*	*f*	*nf*	*f* + *nf*	*f* + *nf*
α-Pinene	28·0	29·2	28·6 (12·0)	18·9	15·2	17·1 (6·5)	20·7	15·2	18·0 (3·6)	56·5	58·3	57·4 (13·2)	30·3
Camphene	0·2	0·1	0·2 (0·2)	0·1	0·1	0·1 (0·1)	0·3	0·5	0·4 (0·1)	0·5	0·7	0·6 (0·2)	0·3
β-Pinene (+Sabinene)	3·1	3·5	3·3 (0·6)	2·9	3·0	3·0 (0·4)	25·7	21·1	23·4 (4·0)	20·9	11·4	16·2 (7·8)	11·5
Δ^3-Carene	54·6	49·4	52·0 (13·3)	69·6	73·1	71·3 (5·7)	38·6	42·8	40·7 (3·1)	0	0	0 (0)	40·9
Myrcene	3·9	6·2	5·0 (2·9)	2·2	2·2	2·2 (0·2)	10·5	16·0	13·2 (3·9)	30·8	28·4	24·6 (10·4)	11·3
α-Terpinene	0·1	0·1	0·1 (0·2)	0·4	0·6	0·5 (0·4)	0·2	0·4	0·3 (0·2)	0	0	0 (0)	0·2
Limonene	0·4	0·4	0·4 (0·1)	0·3	0·3	0·3 (0·1)	0·4	0·4	0·4 (0·1)	0·7	0·6	0·7 (0·1)	0·4
β-Phellandrene	4·3	6·8	5·5 (4·0)	0·3	0·2	0·3 (0·1)	0·7	0·8	0·7 (0·1)	0·6	0·5	0·5 (0·2)	1·8
γ-Terpinene	0·4	0·3	0·3 (0·1)	0·4	0·4	0·4 (0·2)	0·2	0·2	0·2 (0·1)	<0·1	<0·1	<0·1 (0·1)	0·2
Terpinolene	5·1	3·7	4·4 (1·1)	4·6	4·3	4·4 (1·5)	2·3	2·4	2·4 (0·8)	0	0	0 (0)	2·8
Unidentified	<0·1	0·2	0·1 (0·3)	0·4	0·6	0·5 (0·4)	0·3	0·4	0·4 (0·2)	0	0	0 (0)	0·2

Refer to mean values for 6 and 12 sample trees, respectively. The figures in parentheses refer to S.D.. *f* = fertilized plots. *nf* = not fertilized plots.

EXPERIMENTAL

Field trial. The trees sampled were growing in a clone trial, laid out in the State Forest of Lindhof, *ca* 20 km SW of Stockholm. The trial which was initiated by the Departments of Plant Ecology and Forest Genetics, Royal College of Forestry, aimed at studying the interaction between the plant genotype and the environment, including different nutrient levels and spacing.[9,10] Four clones were analyzed differing markedly regarding a number of economically important characters, e.g. growth rate, stem form, branching habit, seed production capacity and seed germination ability.[11] The grafts were grown partly at two different mineral nutrient levels and partly at two different spacings. For determining the monoterpene composition, only the eight plots with a dense spacing were sampled.

Clones: A 2201 Växbo, B 4400 Brännbo, C 4013 Evertsberg, D 5003 Siljansfors

Fertilizer treatments: I not fertilized (nf), II fertilized (f) The fertilizer treatment consisted of an application

[9] ANDERSSON, E. and TAMM, C. O. (1963) *Förbandsförsök med klonplantor av tall på kronoparken Lindhof. Stockholms revir*, Skogshögskolan, Stockholm (unpublished).

[10] HATTEMER, H. unpublished data.

[11] ANDERSSON, E. (1966) *International Union of Forest Research Organizations, Section 22, Special Meeting and Excursion in Yugoslovia*, Zagreb 1965, p. 21.

of 30 g of NPK 10–10–15 per plant in the spring of 1964, and after that a yearly dose of 500 kg/ha of NPK 10–10–15.

Spacing: 1·5 × 1·5 m. The grafts were planted on 29 and 30 April 1963. The total area of the plots was 380 m^2, out of which the sampling area constituted 100 m^2. The experimental treatments were without replications.

Sampling technique. The sampling of oleoresin was carried out in June 1972. With a razor blade the last year terminal shoot was cut off *ca* 2 cm beneath the top. Generally the branches of the 4th whorl from top were used. Within a few min after cutting, a drop of gum exuded at the cut surface of the branch. The exuded gum was transferred to a small test tube and stored in a freezer (at −18°) until analyzed. From each of the eight plots, six individuals were sampled for the chemical analysis, i.e. a total of 48 plants.

Chemical analysis. The determination of monoterpene composition of the oleoresin samples was made by GLC using LKB-9000 GC-MS.

The *operating conditions* were as follows: column, 3 m × 2·5 mm glass column filled with 20% Carbowax-20 M on Chromosorb W-AW-DMCS 80-100 mesh; temp., column 90°, separator 200°, ion source 250°, injection port 275°; carrier gas, helium, 20 cc/min; sample size, 150–450 μg oleoresin (gum) dissolved in *ca* 3 vol. of acetone. The *identification* of the effluents from the gas chromatograph was made on the basis of R_t data, obtained partly from previously published investigations[12,13] and partly from own tests on chemically pure monoterpene samples. In doubtful cases, the MS were measured.[14] It was found that the column used did not separate myrcene and Δ^3-carene. The mass spectra from this unresolved peak showed that, with the exception of clone D, it consisted of a mixture of myrcene and Δ^3-carene. The proportion in which they occurred could be estimated from these.[15] The percentages of the individual constituents were obtained by measuring the area of the corresponding peak and relating it to the total area of the monoterpene peaks. All the clones gave approximately the same total area per g oleoresin. Compared to peak areas of pure monoterpene samples the monoterpene content in oleoresin was estimated to 20–25%.

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