

MONOTERPENE COMPOSITION OF NEEDLE OIL OF *PINUS OOCARPA* AND *PINUS CARIBAEA*

KARI LÖYTTYNIEMI, RIITTA LÖYTTYNIEMI, RAIMO HILTUNEN
and SEPPO RÄISÄNEN

Seloste

PINUS OOCARPA- JA *PINUS CARIBAEA* -MÄNTYJEN NEULASÖLJYN MONOTERPEENIKOOSTUMUS

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The monoterpene composition of the needle oil of *Pinus oocarpa* Schiede and *P. caribaea* Morelet growing in Zambia were studied by means of GC-MS. *P. oocarpa* needle samples were taken from 29 trees representing different clones in a seed orchard and from 43 trees in a commercial plantation in May, 1980. The *P. caribaea* needle samples were collected from 17 clones in a seed orchard. Nine compounds were positively identified in the monoterpene fraction of *P. oocarpa* and eight in that of *P. caribaea*. The amounts of these compounds showed marked tree-to-tree variation and the sample trees were screened into distinct groups on the basis of the variation. The results give a basis for selection in favour of pest resistance and turpentine yield.

INTRODUCTION

The monoterpene composition and tree-to-tree variation in the needle oil of a *Pinus kesiya* Royle ex Gordon provenance from Zambia has been determined as part of a study designed to evaluate the susceptibility of industrial forest plantations to pests in Zambia. The results also throw light on the chemotaxonomy of *P. kesiya* and on the specific character of this pine species as a potential source of commercial turpentine (Hiltunen and Löyttyniemi 1978, Löyttyniemi 1980).

The monoterpene composition of needle oil in *Pinus oocarpa* Schiede, another exotic pine species planted on a commercial scale in Zambia, has also recently been screened. The results of the analyses are reported in this paper. Some preliminary findings concerning the monoterpene composition of *Pinus caribaea* Morelet are also presented.

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MATERIAL AND METHODS

Sample collection

The trees used in the study were growing at the Chati Forest Reserve, Zambia (about S 12°50'; E 27°50'; 1300 m a.s.l.). The *P. oocarpa* needle samples were taken from a seed orchard and from a commercial plantation, and those of *P. caribaea* from a seed orchard, in May 1980.

In the seed orchards, needle samples were taken from one tree in each clone. In the commercial *P. oocarpa* plantation, needle samples were collected from 43 trees chosen randomly throughout the area (27 hectares). Needles were picked from the last fully developed needle whorl on different sides of the trees at a height of about 3 metres.

The *P. oocarpa* seed orchard (Chati, S III-2a) consisted of trees representing 30 clones (Mikkola 1979). However, clone No. 21 was not found during needle sample collection. The orchard was based on grafted material from plus trees growing at Samfya, Zambia (Compartment No. 20). The seed for the commercial plantation (Chati, No. 461) originated from the same compartment at Samfya. According to the records, the ancestor seed stock has been collected from Tegucigalpa, Honduras (see Greaves 1979). When the samples were collected in May 1980, the trees in the orchard were 10 years old, the height averaging 13.5 m and the DBH 16 cm. The trees in the commercial plantation were seven years old, the height and DBH being 9.2 m and 12 cm, respectively.

The *P. caribaea* seed orchard (Chati, S X-2) originally consisted of trees of 20 clones (Mikkola 1979) but only 17 were found during needle sampling (Nos. 7, 8 and 10 were missing). Scions for the orchard have been taken from plus trees growing in Zambia, except Nos. 1, 2 and 5 which were from Malawi. The ancestor seed stock originated from Cuba (Nos. 3, 4, 6), Belize (Nos. 9, 11, 17-20) and Australia (Nos. 12-16). The origin of the Malawian clones is not known (see Greaves 1978). The trees were nine years old in 1980. The mean height and DBH of the study trees were 8.9 m and 12 cm, respectively. The trees were suffering from drought and growing on poor soil.

RESULTS AND DISCUSSION

Green et al. (1974, 1975) and Burley and Green (1977) have screened the turpentine composition of stem oleoresin in several *P. oocarpa* and *P. caribaea* provenances from natural stands in Central America and the Bahamas, and found considerable differences in monoterpene composition between the populations and individual trees. Notes on monoterpene composition of *P. caribaea* needle oil from Nigeria (Ekundayo 1978) and *P. caribaea* stem oleoresin from Fiji (Smith 1975) and from Cuba (Weissmann and Vorher 1975) have also been presented.

P. oocarpa

Nine positively identified compounds were now detected in the monoterpene hydrocarbon fraction of the needle oil of *P. oocarpa* (Tables 1 and 2). Five of the six main compounds (mean > 1%) occurred in all of the 72

Analytical

One to seven needles of *P. oocarpa* or half a needle of *P. caribaea* were cut into 2 mm-long pieces. The terpenes were extracted with 2 ml of n-hexane (HPLC Grade, Rathburn Chemicals Ltd., Peeblesshire, Scotland).

GC separations were carried out on a Free Fatty Acid Phase (FFAP) glass capillary column (70 m × 0.35 mm I.D.) with the following conditions: injection port temperature 250°C, interface temperature 260°C, injection at room temperature. After the solvent had been eluted the oven was programmed from 55°C to 180°C at 4°C/min. The samples were injected using the split sampling technique.

GC-MS analyses were obtained on a Carlo Erba Fractovap 2300 gas chromatograph coupled to a Jeol D 100 mass spectrometer by means of a Pt-Ir-tube (30 cm × 0.30 mm O.D., 0.15 mm I.D.) into the ion source without a helium separator. The MS parameters were as follows: electron energy 30 eV, electron multiplier voltage 1.2 kV, recorder input 0.1 V, ionisation current 300 µA and ion source temperature 220°C. The ion monitor was adjusted to m/z 93 and the resolution to 500.

Quantitative determinations based on peak areas were measured by a Hewlett-Packard 3390 A peak integrator connected to the mass spectrometer.

trees analyzed and made up, on the average, 93 per cent of the total amount of monoterpene hydrocarbons. In the stem oleoresin of *P. oocarpa*, four of these compounds, namely α-pinene, β-pinene, 3-carene and β-phellandrene, have earlier been detected as main compounds (Green et al. 1974, 1975, Burley and Green 1977).

The amounts of each monoterpene compound identified showed marked tree-to-tree variation (Tables 1 and 2), thus indicating large phenotypic diversity between the trees. The variation in α-pinene, β-pinene, 3-carene and β-phellandrene was of approximately the same order of magnitude as in stem oleoresin (Green et al. 1974, 1975, Burley and Green 1977).

The sample trees were screened into eight groups representing distinct types according to the proportions of the main monoterpene constituents in the needle oil (Tables 3 and 4): A = α-pinene type; B = α-pinene/β-

Table 1. Statistical data for nine needle oil monoterpenes from 29 *Pinus oocarpa* clones in a seed orchard. (In per cent of total monoterpenes).

Constituent	Range	Mean	S.E.	Cv (%)
Tricyclene	n.d. ¹⁾ - 0.9	0.21	0.05	137.4
α-pinene	19.6-90.7	68.07	3.35	26.5
Camphene	0.4- 1.7	0.72	0.07	48.7
β-pinene	3.4-49.1	17.81	2.55	77.1
3-carene	n.d.-37.0	3.43	1.87	294.5
Myrcene	0.8- 6.5	1.38	0.19	76.0
Limonene	0.3-43.3	2.81	1.47	282.7
β-phellandrene	0.9-33.9	4.54	1.17	138.4
Terpinolene	n.d.-2.6	0.28	0.13	262.2

¹⁾ n.d. = not detected

Table 2. Statistical data for nine needle oil monoterpenes from 43 *Pinus oocarpa* trees growing in a commercial plantation. (In per cent of total monoterpenes).

Constituent	Range	Mean	S.E.	Cv (%)
Tricyclene	n.d. - 1.1			
α-pinene	21.4-88.2	68.50	2.70	25.8
Camphene	tr. ¹⁾ - 1.9	0.63	0.05	54.9
β-pinene	2.8-34.3	13.67	1.29	61.7
3-carene	tr.-58.3	5.70	2.13	244.7
Myrcene	0.4- 6.5	1.52	0.18	78.2
Limonene	0.1-44.0	2.60	1.05	265.3
β-phellandrene	1.1-41.3	5.37	1.21	147.7
Terpinolene	n.d. - 5.8			

¹⁾ tr. = traces

Table 3. Grouped data for nine needle oil monoterpenes from 29 *Pinus oocarpa* clones. (In per cent of total monoterpenes).

Group Clones, No.	A		B		C ₁	D	E	F	G	H
	Mean	S.E.	Mean	S.E.	Mean	Mean	Mean			
	3,5,6,7,10,12,18,19,24		1,4,8,13,14,15,17,22,23,25,28		2,20	9,29	26,27	11	30	16
Tricyclene	0.36	0.13	0.04	0.02	0.30	0.60	0.15	0.5	n.d.	n.d.
α-pinene	86.41	1.21	63.27	1.84	44.85	73.25	79.40	19.6	31.3	54.5
Camphene	0.93	0.15	0.51	0.04	0.85	0.80	0.70	0.9	0.4	0.9
β-pinene	6.79	0.83	31.01	1.90	5.85	13.25	7.35	49.1	4.1	8.1
3-carene	0.19	0.09	0.09	0.04	36.65	0.15	tr.	22.5	0.6	n.d.
Myrcene	1.06	0.03	1.00	0.03	2.20	1.15	1.45	1.8	6.5	1.5
Limonene	1.31	0.14	0.81	0.08	0.65	6.30	1.10	0.4	43.3	0.9
β-phellandrene	2.04	0.23	3.09	0.39	3.45	2.55	9.05	2.1	13.3	33.9
Terpinolene	n.d.		n.d.		2.05	1.30	n.d.	1.3	n.d.	n.d.

Table 4. Grouped data for nine needle oil monoterpenes from 43 *Pinus oocarpa* trees. (In per cent of total monoterpenes).

Group No. of trees	A		B		C ₁	C ₂ ¹⁾	E	G	H	
	Mean	S.E.	Mean	S.E.	Mean	Mean	Mean	S.E.	Mean	
	16		14		3	2	5	1	2	
Tricyclene	tr.		tr.		tr.	0.25	tr.		1.1	0.45
α-pinene	82.59	0.75	69.05	1.53	44.03	22.10	73.12	1.60	38.3	38.60
Camphene	0.66	0.07	0.56	0.07	0.27	0.55	0.78	0.14	1.9	0.45
β-pinene	9.49	0.39	24.00	1.60	10.93	3.95	7.62	2.72	6.4	7.35
3-carene	0.88	0.21	0.89	0.25	32.87	55.55	0.92	0.55	0.4	1.85
Myrcene	1.16	0.06	0.89	0.06	2.20	3.10	1.36	0.11	6.5	4.20
Limonene	1.20	0.19	1.02	0.25	0.87	2.10	1.52	0.42	44.0	10.00
β-phellandrene	2.54	0.21	2.50	0.25	3.07	3.45	12.92	1.67	1.5	36.55
Terpinolene	tr.		tr.		1.90	4.70	0.70	0.37	n.d.	n.d.

¹⁾ C-group divided into two types

pinene type; C = α -pinene/3-carene type; D = α -pinene/ β -pinene/limonene type; E = α -pinene/ β -phellandrene type; F = β -pinene/3-carene type; G = limonene type; H = β -phellandrene type (cf. Hiltunen and Löytty-niemi 1978).

P. caribaea

Eight compounds in the monoterpene fraction of the *P. caribaea* needle oil were positively identified (Table 5). The composition is characterized by high β -phellandrene contents. 3-carene, which was present in the stem oleoresin, was not now found in the needle oil. On the other hand, tricyclene and sabinene were detected in the needle oil. The α -pinene content of the needle oil was also low compared to that of the stem oleoresin (Green et al. 1974, 1975, Smith 1975, Weissmann and Vorher 1975, Burley and Green 1977, cf. also Iloff and Mirov 1954, Ekundayo 1978).

The 17 sample trees were tentatively grouped into three types on the basis of the tree-to-tree variation in the proportions of different monoterpene compounds (Table 6): A = β -phellandrene/limonene type; B = β -phellandrene type; C = β -phellandrene/limonene/ β -pinene type. This grouping agrees quite well with the geographical origin of the clones.

CONCLUSIONS

The results show that the tree-to-tree variation in the composition of the monoterpene fraction is high within the Samfyan provenance of *P. oocarpa*, which is being planted on a large scale in Zambia today, and that the provenance possibly consists of distinct chemotypes. This offers a good basis for selection in favour of pest resistance and turpentine yield. The trees with high pinene contents, especially, possess a turpentine composition which is favourable for commercial

Table 5. Statistical data for eight needle oil monoterpenes from 17 *Pinus caribaea* clones in a seed orchard. (In per cent of total monoterpenes).

Constituent	Range	Mean	S.E.	Cv (%)
Tricyclene	tr. - 0.5			
α -pinene	5.6-19.2	11.71	0.99	34.8
Camphene	tr. - 0.6			
β -pinene	0.6-21.9	3.87	1.65	175.3
Sabinene	0.2- 1.2	0.71	0.07	39.1
Myrcene	3.2- 7.0	4.85	0.28	24.2
Limonene	0.5-24.4	9.99	1.82	75.2
β -phellandrene	39.3-84.6	68.49	3.05	18.4

Table 6. Grouped data for eight needle oil monoterpenes from 17 *Pinus caribaea* clones. (In per cent of total monoterpenes).

Group Clones, No.	A		B		C
	Mean	S.E.	Mean	S.E.	Mean
	1,2,3,5,9,11 18,19,20		12,13,14,15, 16,17		4,6
Tricyclene	tr.		tr.		0.30
α -pinene	9.38	0.84	13.38	1.64	17.15
Camphene	tr.		tr.		0.25
β -pinene	1.21	0.17	1.88	0.37	21.80
Sabinene	0.73	0.08	0.82	0.10	0.30
Myrcene	5.44	0.39	4.10	0.37	4.45
Limonene	14.98	1.55	1.13	0.21	14.10
β -phellandrene	67.90	2.28	78.40	1.96	41.55

turpentine production, at least of technical foliage (cf. Hiltunen 1976).

The terpene composition of needle oil of *P. caribaea*, a pine species grown only on a pilot scale in Zambia, appeared to be quite different from that of *P. kesiya* and *P. oocarpa* in Zambia, and the potential value of this pine species as a source of marketable foliage turpentine is not very high in the Zambian conditions.

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SELOSTE

PINUS OOCARPA- JA *PINUS CARIBAEA*-MÄNTYJEN NEULASÖLJYN MONOTERPEENIKOOSTUMUS

Pinus oocarpa- ja *P. caribaea*-mäntyjen neulasöljyn monoterpeenikoostumusta tutkittiin Sambiansa kasvavista puista otetuista näytteistä kaasukromatografia-masspektrometriamenetelmällä. Yhteensä analysoitiin 72 *P. oocarpa*- ja 17 *P. caribaea*- näytettä.

P. oocarpa-männyn monoterpeenifraktiosta tunnistet-

tiin yhdeksän aineosaa ja *P. caribaea*-männystä kahdeksan. Näiden aineosien pitoisuuksissa oli puuyksilöiden välillä huomattavaa vaihtelua ja puut voitiin ryhmitellä tämän vaihtelun perusteella. Tulokset antavat perustietoa valinnan suorittamiseksi tuholaiskestävyyden ja markkinointikelpoisen öljyn tuottamisen kannalta.