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MONOTERPENES IN SITKA SPRUCE: WITHIN TREE AND SEASONAL VARIATION

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Abstract—The composition of oils from needles and cortex of Sitka spruce is unique. Cortex oil is essentially all monoterpene hydrocarbons, while needle oil may be up to 50% oxygenated monoterpenes. Very wide seasonal variations in composition occur in needle oil in young tissue. At bud burst, the oil is >95% myrcene; this drops to about 40% at the end of summer. The oxygenated terpenes camphor and piperitone develop to about 20% each in concentration during the growing season.

INTRODUCTION

THE COMPOSITION of foliage oil from Sitka spruce (*Picea sitchensis* (Bong.) Carr.) was reported initially by Lynn and Lehman[1] and more recently by von Rudloff.[2] Monoterpenes which occur in this oil are summarized in Table 1; these materials have now been verified by mass spectrometry. Unusual features of this conifer oil are the relatively high (~45%) amount of oxygenated monoterpenes and the combination of myrcene, piperitone and camphor as the main components. The present study of the oil has been done to define the seasonal and within tree variations in the oil which may influence the host selection and oviposition behavior of the Sitka Spruce Weevil *Pissodes strobi* (Peck).[3]

TABLE 1. SITKA SPRUCE MONOTERPENES

α-Pinene	Linalool
Camphene	Terpene alchol (1)
β-Pinene	Bornyl acetate
Myrcene	Terpinene-4-ol
α-Phellandrene	Terpene alcohol (2)
1,4-Cineole	Citronellyl acetate
Limonene	α-Terpineol
β-Phellandrene	Borneol
1,8-Cineole	Piperitone
γ-Terpinene	Geranyl acetate
Terpinolene	Citronellol
Camphor	Geraniol

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[1] LEHMANN, A. J. and LYNN, E. V. (1931) *J. Am. Pharm. Assoc.* **20,** 29.

[2] VON RUDLOFF, E. (1964) *Can. J. Chem.* **42,** 1057.

[3] GARA, R. I., CARLSON, R. L. and HRUTFIORD, B. F. (1971) *Ann. Ent. Soc. Am.* **64,** 467.

The resin canal system in Sitka spruce needles, cortex, phloem and xylem in tissue of up to 3-yr-old have been examined in some detail recently.[4] An extensive system of branched canals is found in the cortex, this system extends up to, but does *not* connect with, the canal system in the needles. This is similar to the situation in *Pinus pinaster*.[5] No resin canals were observed in young phloem and xylem tissue does not have resin canals.

The damage done by the Sitka spruce weevil is predominately in the terminal from the previous year and for this reason extensive sampling was done in cortex tissue in developing terminals and laterals. Other tissue up to seven years in age was also collected. The insects avoid needles and rarely spend much time on the new growing leader; they oviposit near the top of the previous year's terminal. The insects almost certainly encounter resin canals,[6] and the monoterpenes may be involved in this localized oviposition behavior. Monoterpenes released from host species recently have been implicated in insect attraction[7,8] and evidence is accumulating that they are involved in attracting insects closely related to the Sitka Spruce weevil[9,10] to their host. Evidence for the existence of a pheremone has recently been reported.[11]

RESULTS AND DISCUSSION

Needle oil and cortex oil

The results of the analysis of oils from foliage and cortex of leaders for the principal monoterpene components are summarized in Table 2. The needle oil composition found is similar to that reported by von Rudloff[2] i.e. high in myrcene, camphor and piperitone. Analysis of several cortex oils shows that these are made up of mostly α- and β-pinene and β-phellandrene. The oils from these two locations are very different in composition; essentially the cortex oil is hydrocarbon in nature, while the foliage oil is about 45% oxygenated monoterpenes. The differences are especially apparent in the September samples which are from the same trees.

TABLE 2. MONOTERPENE COMPOSITION OF NEEDLE OIL AND CORTEX OIL FROM SITKA SPRUCE

Oil source	α-Pinene	β-Pinene	Myrcene	Limonene	β-Phellandrene	Camphor	Piperitone
Foliage*	1·5	0·8	23·5	0·9	7·1	17·2	23·1
Needles†	1·9	1·1	30·0	2·8	11·2	18·0	28·1
Cortex†	22·3	13·7	14·5	11·7	37·7	tr	tr
Cortex‡	13·4	17·7	13·0	3·8	41·6	2·0	6·4

* Data of von Rudloff.[2]
† Data from two trees averaged, collected September 1969.
‡ Data from five trees averaged, sampled February 1969.

Effect of location on needle oil composition

Needle oil from various locations in the tree was examined in samples collected in Feb. 1970, and the results of these analysis are shown in Table 3. The oil from main stem needles

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of 1–4 yr is in general typical of needle oil, i.e. around 20% in myrcene, camphor and piperitone and with only traces of α- and β-pinene and about 10% β-phellandrene. As the age of the oil increases, there appears to be a trend towards lower myrcene and higher piperitone content. Oils isolated from needles 1 and 3 yr of age taken from laterals 3- and 7-yr-old respectively were also analyzed; these oils are similar in composition and are also similar to the oil from needles on the main stem, with the exception that they contain about twice as much myrcene and about 20% less total oxygenated monoterpenes.

TABLE 3. NEEDLE OIL COMPOSITION AT VARIOUS LOCATIONS

Location	Needle age (yrs)	α-Pinene	Camphene	β-Pinene	Myrcene	Limonene	β-Phellandrene	Camphor	Piperitone
Main stem	1	2·0	1·6	1·3	17·9	2·9	11·1	25·3	29·9
	2	3·9	2·7	2·1	28·0	3·3	11·1	15·2	22·9
	3	1·1	0·5	1·0	14·1	1·7	8·4	26·0	38·0
	4	0·96	0·86	0·89	18·9	2·4	10·1	20·7	34·1
3-yr-old lateral branch	1	2·0	2·3	1·1	31·5	2·7	11·3	14·2	24·5
	3	2·8	2·6	1·2	37·8	3·2	11·7	18·9	23·0
7-yr-old lateral branch	1	1·3	1·1	0·9	33·6	2·6	14·4	10·3	25·2
	3	1·2	1·4	0·8	28·7	2·5	13·5	13·3	27·2

Effect of location on cortex oil composition

Oil from cortex tissue at various locations in the tree also was examined and the results are summarized in Table 4. The main stem cortex oil is similar to that reported from a different tree composite in Table 2 and does not show any trend with increasing tissue age except possibly a slow conversion of myrcene to α-pinene, probably a non-enzymatic reaction. Oil from cortex tissue of laterals does vary with lateral age; in young lateral tissue the oil is nearly identical to main stem cortex oil and the oil becomes nearly identical to needle oil with increasing lateral age, i.e. the same oxygenated terpenes are present in amounts around 40%.

TABLE 4. COMPOSITION OF CORTEX OIL FROM SEVERAL LOCATIONS IN THE TREE

Location	Tissue age (yrs)	α-Pinene	β-Pinene	Myrcene	Limonene	β-Phellandrene	Camphor	Piperitone
Mainstem	1	19·2	16·6	14·2	10·0	38·6	< 1	< 1
	2	20·1	18·2	13·0	9·6	38·9	< 1	< 1
	4	23·8	17·1	9·6	9·4	40·2	< 1	< 1
	7	25·5	15·3	7·8	12·0	39·8	< 1	< 1
3-yr-old lateral branch	1	6·4	9·7	18·8	1·6	39·8	5·3	15·0
7-yr-old lateral branch	1	4·2	3·5	18·1	1·7	24·6	—	34·5

Initially we were working primarily with oil from new and 1-yr-old leaders and were puzzled by the rather large differences in composition from that reported by von Rudloff, whose results were based on whole branchlets which would contain both cortex and needle oil. In view of the above results this is easily explained, since most of von Rudloff's samples are taken from older branches near the bottom of larger trees, where needle and cortex oil are essentially identical.

TABLE 5. SEASONAL VARIATION IN OIL COMPOSITION OF NEW NEEDLES THROUGH 2 yr GROWTH

Date	α-Pinene	Camphene	β-Pinene	Myrcene	Limonene	β-Phellandrene	Camphor	Piperitone	Other
4-25	1	tr	tr	93	5	1	0	0	0
5-20	2	0	2	93	tr	3	0	0	0
5-29	1	tr	tr	94	3	1	0	0	1
6-9	2	tr	1	84	2	6	0	2	3
6-21	1	tr	1	78	6	7	tr	5	2
7-6	4	2	2	61	2	10	4	10	5
7-19	4	2	3	44	2	11	7	13	14
9-9	3	2	2	39	3	12	8	15	16
10-1	3	2	2	35	4	12	11	18	13
4-19	4	2	2	42	6	12	11	13	8
4-25	6	1	7	37	3	20	5	12	9
5-3	7	2	4	38	16	15	7	10	1
5-16	1	0	1	37	2	18	4	20	17
6-9	3	1	2	42	2	17	2	30	1
7-6	3	2	2	43	2	11	8	22	7
7-19	5	4	2	40	2	7	7	7	26

Seasonal variation

Samples of leaders were obtained throughout the 1970 season both of the 1969 terminal and of the new terminal produced in 1970. The results of the needle oil analysis are shown in Table 5. The upper part of the Table is from the needles of new 1970 growth and the lower part is from the 1969 growth taken at the same time. Of major interest is the observation that the initial oil formed consists almost exclusively of myrcene with no detectable oxygenated compounds. The myrcene content of the oil declines rapidly during the growing season. The other significant changes are an active increase in the β-phellandrene concentration as well as increases in camphor and piperitone. In their second year, needles do not show a marked change in myrcene content with the onset of new growth, piperitone does however pass through a marked peak, while camphor drops in concentration and then increases again. These changes in composition i.e. very large change in myrcene content, are similar to the changes in white spruce monoterpenes recently reported by von Rudloff, who has given an excellent review of this subject.[12] Cortex oil in the leaders does not undergo any change during the growing season.

EXPERIMENTAL

Samples for the within-tree variation and seasonal variation studies were obtained from a 20-yr-old Sitka Spruce plantation located near Cathlamet, Washington. The spruce stands ranged in height from 5 m on drier sites to over 8 m on the lower slopes. Sampling was done after bud closure in Sept. 1969 and during the winter in Feb. 1970. Needles and cortex samples from 1-, 2-, 4- and 7-yr-old main stem and lateral branches were taken from trees of average age 15 yr. Sampling for the seasonal variation study was done from April–Sept. 1970. Both new growth and the previous seasons growth on the main stem were collected. Also, samples of new growth were taken on laterals. Samples were usually composites from 2 to 5 trees collected at about 2 week intervals.

The monoterpenes were recovered from the tissue by steam distillation. Needles were carefully removed from the buds and developing stem and these and the cortex tissue were distilled separately. The recovered oils were analyzed by GLC using a 100′ Carbowax 20 M S.C.O.T. column. Most analysis were done by temp programming from 90–140° at 2·5°/min. A 50′ UCON HB 2000 S.C.O.T. column and a 150′ β-β-oxydipropionitrile open tubular column were used for compound verifications. Identity was done by comparison of relative retention times with known compounds, by use of GLC in combination with hydrolysis and acetylation reactions and by MS comparisons with known compounds.

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