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Volatile oil analysis of spruce galls caused by *Sacchiphantes viridis* Ratz.

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Abstract

The results of the analysis of volatile oil obtained from galls caused by *Sacchiphantes viridis* are presented in this paper. It was found that galls contain more oil than normal shoots. The composition of the oil obtained from galls differs significantly from that of normal shoots. The differences observed in the amount of camphene, β-pinene, myrcene, 3-carene, unidentinfied compounds 14 and 17, camphor, fenchyl alcohol, borneol and citronellol seem to be connected with the deterioration of central resin canals in the stem cortex during the development of galls and with the formation of new and very numerous canals in the bases of the differentiated gall leaves.

Key words: *volatile oil, spruce gall*

INTRODUCTION

Spruce galls have been the object of considerable interest because of the changes undergone by developing shoots under the influence of gall aphids. Despite detailed anatomical and cytological studies, relatively little attention has been focused on the growth of resin canals in galls. Some of the changes have been described by Cosens (1912), Küster (1930) and Rohfritsch (1966).

Recently, changes within the secretory tissue of *Picea abies* were analysed taking as an example, galls caused by *Sacchiphantes viridis*

(Miszta 1983, 1984, 1986). It was demonstrated that during the growth of the galls, first the central resin canals in the stem cortex and canals normally present in leaves deteriorate then very fine and numerous resin canals form under the epidermis in the area of the swollen bases of leaves.

Electron microscopic observations revealed that epithelial cells of the transformed resin canals in the stem cortex were similar to the cells of the nutritive tissue described by Rohfritsch (1977), whereas cells of new canals in the bases of leaves were normal epithelial cells whose structure indicated an intense but short secretory activity (Miszta 1986).

Stages of changes observed during the development of resin canals correlated positively not only with the phases of development of spruce vegetative shoots but also with the phases of gall development. In general, the central resin canals deteriorate before the larvae move to the cavities formed by the swollen bases of leaves and new canals in the leaves form numerously after this period. In accordance with this, differences in the composition of volatile oil obtained from unchanged shoots and from galls were expected. The results of the analysis are presented in this work.

MATERIALS AND METHODS

In 1982, one-year-old shoots were collected from 15–20 year old *Picea abies* (L.) Karst. trees growing in the Repecko Forest Inspectorate. The shoots were collected during the period of intense development of galls, always from the same ten unattacked trees and ten trees attacted by *Sacchiphantes viridis* Ratz. by more than 50%, taking into account the number of terminal buds transformed into galls. Observation revealed that lateral galls developed when one fundatrix inhabited the bud, therefore galls induced by two or more fundatrices were chosen for analysis.

Collection periods were according to stages of gall development and changes in the system of resin canals. The first period was shortly before emergence of larvae from the eggs layed by the fundatrix. During this period, deterioration of central resin canals in the stem cortex was observed within the galls. During the second period, after the larvae moved to the cavities, numerous resin canals formed in the swollen bases of gall leaves. The third period, was while the larvae were still inside the cavities, but structural signs of organelle disintegration and decrease of secretory activity were noted in the epithelial cells in galls.

The material collected was transported in containers with dry ice and stored in the freezer compartment of a refrigerator. Prior to analysis, it was divided into four groups denoted as follows: N — shoots of unattacked trees, n — normal shoots of trees attacked by *S. viridis*, G — galls, pg — shoots with a normal structure but growing from the top of galls.

Firstly, the percentage of oil respect to the dry weight of shoots was determined by means of steam distillation. Next, the oil was analysed in a ICSO 574 H gas chromatograph equipped with a flame-ionization detector. A steel column, 3.3 m long with a 4 mm internal diameter packed with Chromosorb W NAW 80/100 and coated with 20% Carbowax 20 M was used. The flow rate of argon was 25 cm^3 min^{-1}, detector temperature was 240°C, the injection port temperature was 280°C, the oven temperature was programmed to increase at 10° min^{-1} from 100 to 200°C, sample size was 5 mm^3.

Taking into account that the differences observed could be related to the seasonal variation of oil composition, reported by Von Rudloff (1967, 1972, 1975a, b) and Forrest (1980) for the species *Picea*, the results obtained were statistically analysed (by the t-test) in two ways. First, the significance of differences between means from the whole tested period was calculated for four kinds samples, i.e. shoots of trees unattacked by *S. viridis*, normal shoots of trees attacked, galls and gall shoots. Next, the significance of differences between means obtained from the values for all samples at three subsequent dates, i.e. June 2, June 16 and July 1, 1982 was calculated. The results of these calculations are presented in Table 3.

RESULTS AND DISCUSSION

Table 1 shows the percentage yield of oil from one-year-old shoots of trees unattacked and attacked by *S. viridis*, during the three subsequent stages of gall development. During the first stage, gall shoots were not tested as they were not yet fully developed. The results obtained showed

Table 1

Percentage of oil in one-year-old shoots of spruce trees unattacked and attacted by *Sacchiphantes viridis*

	Trees unattacked	Trees attacked by *S. viridis*		
	normal shoots (N)	normal shoots (n)	galls (G)	gall shoots (pg)
June 2, 1982	0.25	0.40	0.61	–
June 16, 1982	0.45	0.30	0.62	0.46
July 1, 1982	0.62	0.47	0.81	0.66
$\bar{x}$	0.44*	0.39*	0.68*	0.56

* Statistical significance of differences, level of probability = 0.05

that galls contained about twice the amount of oil than normal shoots. The differences were statistically significant for the mean from the whole period tested. The difference between the average yield of oil from galls and the yield of oil from gall shoots was smaller and statistically insignificant.

At the time of chromatographic analysis (results presented in Table 2) 23 peaks were noted on chromatograms for all samples. The peaks were identified by comparison with 21 available standards. Myrcene and 3-carene, camphor and fenchyl alcohol as well as bornyl acetate and terpinene-4-ol were present jointly.

The amount of individual components calculated as a relative percentage share of each was different in the subsequent stages of gall development as well as in individual samples.

In general, galls contained more β-pinene, myrcene together with 3-carene and terpinolene and less camphene, an unidentified compound denoted by number 17, camphor together with fenchyl alcohol and borneol, as compared to normal shoots of trees both unattacked and attacked by *S. viridis*.

During the first stage, a large amount of citronellal in the galls was a characteristic feature.

The comparison of galls with normal shoots (Table 3) revealed statistically significant differences in the case of camphene, β-pinene, myrcene together with 3-carene, unidentified compounds 14 and 17, camphor together with fenchyl alcohol, borneol and citronellol. Changes in the amount of these compounds seem to be a result of the specific development of the secretory tissue in spruce galls.

At subsequent sampling times, significant differences were noted for tricyclene, limonene, β-phellandrene, p-cymene, terpinolene, unidentified compounds 13 and 17, bornyl acetate together with terpinene-4-ol and terpineol. With the exclusion of compound 17, this group of compounds has not been mentioned before and the changes of their amounts seem to be merely the result of seasonal variations observed normally in the course of the development of spruce shoots.

Only changes in the composition of oil connected with the development of spruce galls were analysed in this work. It seems that in the future it would be interesting to determine if there is a relationship between the composition of oil of individual trees and their susceptibility to gall aphids attack.

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Table 2

Relative percentage of oil components of one-year-old spruce shoots unattacked and attacked by *Sacchiphantes viridis* during the period of intense gall development

No.	Component	June 2, 1982			June 16, 1982				July 1, 1982			
		N	Z, n	Z, G	N	Z, n	Z, G	Z, pg	N	Z, n	Z, G	Z, pg
1	santene	trace	0.06	trace	–	–	–	–	–	–	0.12	–
2	tricyclene	1.00	0.95	0.73	0.36	0.29	0.14	0.29	0.51	0.29	0.74	1.41
3	α-pinene	8.38	9.45	9.50	10.56	10.15	11.39	14.88	8.41	9.18	9.53	9.98
4	camphene	9.59	8.69	8.68	13.30	12.22	6.25	12.40	10.18	11.98	5.77	12.04
5	β-pinene	11.68	9.21	12.75	6.89	6.34	18.45	8.85	7.09	8.31	17.26	7.73
6, 7	myrcene +3-carene	11.24	10.49	15.12	9.18	7.58	20.01	10.47	8.95	12.28	14.45	13.23
8	limonene	6.41	10.97	6.78	23.76	19.26	16.24	19.26	10.12	9.58	13.36	9.37
9	β-phellandrene	8.75	9.67	9.58	6.93	5.41	3.25	4.30	8.01	7.40	8.61	7.49
10	p-cymene	0.54	1.96	1.12	0.66	0.55	0.97	0.32	1.46	1.37	2.75	1.17
11	terpinolene	3.17	3.59	3.91	1.25	1.34	3.45	1.13	2.06	2.96	6.76	3.12
12	undetermined	0.17	0.59	trace	0.08	trace	trace	trace	0.83	0.69	trace	0.19
13	undetermined	0.27	0.28	0.13	0.22	trace	0.92	trace	0.56	0.34	0.65	0.64
14	undetermined	0.18	0.47	0.68	trace	trace	0.74	trace	0.63	0.21	0.90	1.53
15	citronellal	0.48	1.03	5.37	1.77	1.64	3.75	0.31	1.14	0.32	0.37	0.81
16	linalool	1.29	4.59	0.97	1.80	1.82	1.59	1.17	1.67	1.12	1.43	1.40
17	undetermined	1.24	2.04	1.13	2.85	2.49	1.24	3.57	4.15	3.14	1.39	3.85
18, 19	camphor +fenchyl alcohol	11.26	7.58	6.53	9.24	10.47	4.29	11.25	10.81	11.00	5.54	10.73
20, 21	bornyl acetate +terpinene-4-ol	4.70	4.63	3.13	1.63	3.64	1.59	2.90	2.25	1.12	1.72	2.17
22	estragole	6.78	4.83	7.00	3.58	7.68	3.67	3.58	5.48	4.17	3.50	4.98
23	borneol	4.90	3.79	2.95	3.86	6.55	1.57	2.96	6.73	7.69	2.51	5.28
24	citronellol	2.69	2.00	1.31	trace	1.71	trace	0.79	2.00	2.01	0.98	1.29
25	α-terpineol	4.22	2.89	1.99	2.10	0.68	0.49	1.56	5.92	4.02	1.24	1.59
26	undetermined	1.03	0.21	0.43	trace	0.17	trace	trace	1.04	0.82	0.39	trace

N – shoots of the trees unattacked, Z – shoots of the trees attacked by *S. viridis*: n – normal shoots, G – galls, pg – gall shoots.

Table 3

Statistical significance of differences between percentage means of oil components from one-year-old shoots of spruce trees unattacked and attacked by *Sacchiphantes viridis*

No.	Component	N / Z. n	Z. n / Z. G	Z. G / Z. pg	N / Z. G	N / Z. pg	Z. n / Z. pg	June 2, 1982 / June 16, 1982	June 16, 1982 / July 1, 1982	June 2, 1982 / July 1, 1982
1	santene	–	–	–	–	–	–	0.29	0.50	0.13
2	tricyclene	0.34	0.10	0.63	0.29	0.47	0.68	4.77*	1.62	0.52
3	α-pinene	0.60	0.70	1.15	1.09	1.62	1.15	2.36	2.25	0.38
4	camphene	0.06	2.78*	4.55*	2.78*	0.79	0.85	0.52	0.13	0.66
5	β-pinene	0.33	4.18*	3.42*	3.22*	0.13	0.29	0.31	–	0.38
6, 7	myrcene + 3-carene	0.21	2.90*	1.90	3.57*	1.48	0.85	0.13	0.14	0.03
8	limonene	0.02	0.28	0.47	0.22	0.12	0.19	5.29*	5.04*	1.56
9	β-phellandrene	0.31	0.15	0.41	0.37	1.08	0.67	4.45*	3.51*	3.45*
10	p-cymene	0.65	0.45	1.08	0.99	0.21	0.87	1.16	2.79*	0.83
11	terpinolene	0.54	1.69	1.61	1.55	0.03	0.40	2.60*	1.66	0.16
12	undetermined	0.22	1.95	1.43	1.50	0.84	1.10	1.21	0.78	0.64
13	undetermined	1.00	1.38	0.58	0.85	0.09	0.33	0.26	1.13	3.80*
14	undetermined	0.17	3.18*	–	2.38	0.81	0.90	1.67	2.25	1.09
15	citronellal	0.25	1.41	1.35	1.33	1.12	0.85	2.13	1.67	1.88
16	linalool	0.86	1.10	0.16	1.18	1.76	1.15	0.59	1.58	0.75
17	undetermined	0.22	4.09*	14.47*	1.79	0.89	2.80*	1.73	0.88	2.77*
18, 19	camphor + fenchyl alcohol	0.62	3.33*	6.30*	5.67*	0.71	0.94	0.16	0.35	0.56
20, 21	bornyl acetate + terpinene-4-ol	0.19	0.84	0.57	0.67	0.26	0.43	3.28*	1.13	4.40*
22	estragole	0.20	0.53	0.28	0.38	0.76	0.86	1.18	0.09	2.14
23	borneol	0.59	2.98*	2.28	2.82*	1.04	1.18	0.11	1.21	1.18
24	citronellol	0.43	2.95*	0.52	0.88	0.49	4.83*	2.36	1.96	0.93
25	α-terpineol	1.05	1.21	0.62	2.37	1.76	0.75	2.58*	1.70	0.11
26	undetermined	0.73	0.50	1.50	1.14	1.57	1.48	2.08	2.17	–

N – shoots of the trees unattacked. Z – shoots of the trees attacked by *S. viridis*: n – normal shoots. G – galls. pg – gall shoots.

* Significance tested using t-test. level of probability = 0.05.

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Analiza olejku eterycznego galasów świerkowych tworzonych przez Sacchiphantes viridis Ratz.

Streszczenie

W pracy przedstawiono wyniki analizy olejku otrzymanego z galasów tworzonych przez *Sacchiphantes viridis*. Stwierdzono, że w galasach znajduje się więcej olejku niż w pędach normalnych. Skład olejku otrzymanego z galasów różni się istotnie od składu olejku z pędów normalnych. Różnice obserwowane w ilościach kamfenu, β-pinenu, mircenu, 3-karenu, niezidentyfikowanych składników 14 i 17, kamfory, alkoholu fenchylowego, borneolu i citronelolu wydają się być związane z zanikaniem, w czasie rozwoju galasów, centralnych przewodów żywicznych miękiszu korowego oraz z powstawaniem nowych i bardzo licznych przewodów w nasadach zmienionych liści galasów.