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THE ESSENTIALS OIL OF *PONCIRUS TRIFOLIATA* (L.) RAF. AND ITS SELECTIONS IN RELATION TO CLASSIFICATION*

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Abstract—A gas–liquid chromatographic analysis of the essential oils of *Poncirus trifoliata* (L.) Raf. was made to determine the percentage of such oils in the rind, pulp and leaf of *Poncirus*, to ascertain the variation that exists within and between strains of *Poncirus*, and to study the changes in the essential rind oil composition occurring during maturation.

INTRODUCTION

Poncirus trifoliata (L.) Raf. shows resistance to nematodes, several diseases and frost. It has often been used as a rootstock and as a parent in crosses with *Citrus*. Because of its commercial importance the authors have undertaken an evaluation of the essential oils of this species to ascertain if they might establish a basis for further taxonomic studies in the sub-family Aurantioideae of the family Rutaceae to which the genus belongs.

Long-continued natural crossings between species, as well as reproduction by asexual embryos, have left *Citrus* taxonomy full of complexities and in a controversial state. One of the most widely used systems of classification of the genus *Citrus* is that by Swingle[1] who lists sixteen species. On the other hand, Tanaka[2] recognizes about 157 species. Hodgson[3] is somewhat intermediate and lists thirty-six species.

During the last 20 years, new biosystematic procedures have become of interest in plant classification. Various types of chromatography have become an integral tool of plant research and have been shown to be useful in assisting taxonomists to obtain comparative data based upon chemical groupings or characters. Until recently, chemical constituents in plants have been investigated only haphazardly; usually the search has been directed towards useful substances for commercial purposes. Consideration of certain chemical characters as an aid in *Citrus* classification could result in a better understanding of the species concept in *Citrus* and in its related genera.

In the plant kingdom, taxonomic attention has been focused primarily on such specific substances as alkaloids, tannins, pigments and essential oils. In recent years, gas chromatographic separation of terpenes has proved valuable in taxonomic studies. Mirov[4] studied the terpenes of *Pinus* and von Rudloff[5,6] the essential oils of *Juniperus*, and phylogenetic

* Paper No. 1707, 1966, University of California, Citrus Research Center and Agricultural Experiment Station.

1 W. T. SWINGLE, *The Botany of Citrus and its Wild Relatives of the Orange Subfamily*. University of California Press, Berkeley (1943).
2 T. TANAKA, *Citrologia*. Semi-centennial Commemoration Papers on Citrus Studies (1961).
3 R. W. HODGSON, *Calif. Citrograph* **46** (8), 246, 256 (1961).
4 N. T. MIROV, *Ann. Rev. Biochem.* **17**, 521 (1948).
5 E. VON RUDLOFF, *Can. J. Chem.* **41**, 2876 (1963).
6 E. VON RUDLOFF, *Can. J. Chem.* **42**, 1057 (1964).

aspects in the genus *Picea*. Gas chromatographic analysis for chemotaxonomic purposes relating to the genus *Abies* were also reported by Zavarin and Snajberk.[7] Other workers in the field have tried to find genetic relationships based upon the chemical composition of the essential oils.[8]

Preliminary work done by the authors has shown that essential oils might prove to be useful as an aid in *Citrus* classification. Chemical differences in species and varieties have been recognized; even strains and F_1 hybrids have been studied. While these chemical differences were occasionally qualitative at the higher taxonomic levels, they were usually quantitative as far as the lower levels were concerned. "Fingerprinting" of the essential oils could therefore provide an approach to problems of rootstock identification, to the separation of nucellar and zygotic seedlings, and to the determination of probable parentage if just one parent and the hybrid are known.

Trees derived from budding on trifoliate orange rootstock are often variable in size. While dwarfing may in some cases be due to the presence of virus diseases, there is some uncertainty as to whether extreme dwarfing or extreme vigor is the result of propagation upon zygotic seedlings. Since trifoliate orange characters are usually dominant, it has been difficult on morphological characters alone to determine whether the rootstock in question is a trifoliate orange or a hybrid.

The purpose of this study has been to explore the possibilities of using gas–liquid chromatography as a tool in the identification of typical *Poncirus* essential oil characteristics by: (*a*) describing typical *Poncirus* essential oils, (*b*) determining the natural variation within and between strains of *Poncirus*, and (*c*) laying the groundwork for future studies involving intergeneric hybrids between *Citrus* and *Poncirus*. No previous work on the essential oil composition of *Poncirus* is known to the authors.

MATERIALS AND METHODS

In order to describe *Poncirus* essential oil characteristics, it was necessary to determine: (*a*) the composition of the oils in different organs of the same plant i.e. in leaves, rind and pulp; (*b*) the chromatographic characteristics of rind oils from different selections; (*c*) the variability that exists within a given selection; and (*d*) the change of the essential oil composition during fruit maturation.

Tissue Sources of Oil

Oils from the leaves, fruit rind and fruit pulp of the Webber–Fawcett strain of trifoliate orange were extracted and used as representative *Poncirus* essential oils. The leaf oils were obtained from about 400 typical, mature leaves harvested from several trees of the same clone. The wet-ground (blended) composite sample was steam-distilled in a Clevenger apparatus to which was added carbon dioxide to prevent oxidation of the oil components. Samples of ten fruits from each of several trees were harvested and composited, and the outer peel (flavedo), without the inner peel (albedo), was wet-ground and steam-distilled. All oils obtained were stored in small vials at 5° under an atmosphere of nitrogen.

Rind Oils of Typical Strains

Rind oils of ten selected strains of *Poncirus*, both small-flowered and large-flowered, were obtained from composite samples of ten fruits taken from the north as well as south sides of

[7] E. ZAVARIN and K. SNAJBERK, *Phytochem.* **4**, 141 (1965).
[8] A. J. HAAGEN-SMIT, *Ann. Rev. Plant Physiol.* **4**, 305 (1952).

TABLE 1. THE ESSENTIAL OILS OF THE LEAVES, RIND, AND PULP OF WEBBER–FAWCETT TRIFOLIATE ORANGE (*Poncirus trifoliata* L.)

Essential oil components		% Relative concentrations of individual components					
		Leaf		Rind		Pulp	
Peak No.	Probable identity	Relative retention time*	Composition (%)	Relative retention time	Composition (%)	Relative retention time	Composition (%)
1	Air					0·16	0·52
2						0·22	0·52
3		0·28	0·02	0·26	0·01		
4		0·38	0·01				
5	Acetone	0·51	0·05	0·47	0·03	0·43	9·21
6				0·57	0·01	0·56	1·43
7				0·67	0·01		
8		0·76	0·02	0·77	0·01		
9				0·86	0·04	0·85	0·38
10	α-Pinene	1·00	0.56	1·00	1·16	1·00	0·44
11						1·16	0·33
12	Camphene	1·38	0·04	1·36	0·07	1·29	0·25
13				1·57	2·92	1·58	0·19
14	β-Myrcene	1·64	7·39	1·68	20·39	1·72	0·35
15	Phellandrene	1·91	1·69	1·95	3·82	2·02	0·12
16	D-Limonene	2·20	1·02	2·24	40·71	2·29	1·80
17	γ-Terpinene	2·40	63·69	2·48	13·49	2·51	1·63
18	Cineol	3·22	(area included in 17)	2·88	0·30		
19	*p*-Cymene	3.46	0·58	3·26	1·94	3·23	1·45
20	Octanal	0·38	0·07	0·40	0·81	0·37	0·26
21		0·41	0·06			0·42	0·36
22	Methyl heptanone	0·48	0·08	0·49	0·13	0·49	0·50
23		0·53	0·04	0·54	0·09	0·56	0·20
24	Nonanal	0·59	0·09	0·59	0·38	0·60	1·64
25		0·67	0·06	0·64	0·32	0·64	0·98
26				0·68	0·06	0·68	0·36
27	Octyl acetate	0·72	0·37	0·76	0·40	0·75	0·36
28	Furfural	0·79	0·09			0·78	1·11
29	Decanal					0·83	0·31
30	Octanol	0·91	0·14	0·90	0·12	0·92	0·47
31	Linalool	1·00	0·12	1·00	0·70	1·00	12·99
32		1·11	0·06			1·11	0·88
33	Nonyl acetate	1·19	0·10	1·19	0·12	1·22	0·43
34	Isopulegol	1·28	0·65	1·31	0·12	1·32	0·70
35	Nonanol	1·40	10·42	1·41	1·65	1·41	9·81
36	Neryl formate	1·52	0·62	1·51	1·85	1·53	0·49
37		1·58	0·61	1·58	0·60	1·60	0·22
38		1·69	0·26				
39	Citronellyl acetate	1·80	0·57	1·82	0·33	1·73	0·50
40	Neryl acetate					1·83	0·43
41		1·92	1·23	1·95	0·25	1·93	2·00
42		2·00	0·62			2·04	0·17
43				2·11	0·98	2·12	0·17
44	α-Terpineol or geranyl formate	2·17	0·63	2·18	0·86	2·22	1·69
45	Citronellal	2·30	0·82	2·28	2·36	2·32	0·21
46		2·39	0·07	2·45	0·17		
47	Neral			2·55	0·09	2·48	0·66
48	Geranyl acetate	2·65	0·94	2·72	0·43	2·70	0·54
49	Geranial	2·86	0·69	2·93	0·21	2·93	0·40
50		3·07	0·66				
51	Nerol	3·43	2·11	3·46	0·57		
52	Geraniol	0·46	0·30				
53		0·50	0·16				
54		0·54	0·49	0·53	0·03		
55		0·63	0·18	0·62	0·09	0·64	1·08
56		0·70	0·34			0·73	3·41
57		0·82	0·31			0·81	3·72
58		0·91	0·08	0·91	0·16	0·94	4·77
59		1·00	0·87	1·00	1·24	1·00	26·39
60		1·08	0·04			1·07	3·21

* Retention time relative to peak numbers 10, 31, and 59.

three trees of each selection. The flavedo was blended and the oils steam-distilled and stored in the manner previously described. Ontogenetic rind oil samplings were made from two (large-flowered Pomeroy and small-flowered Rubidoux) strains by harvesting ten fruits from the north and south sides of three trees of each of these strains. The sampling was repeated on two more harvest dates. These thirty-six fruit samples were blended, the oils steam-distilled and stored in cool temperature until used. The oils were analyzed by gas–liquid chromatography (GLC) for their content of eighteen prominent components (peaks). soon after distillation in order to minimize storage time as a variable.

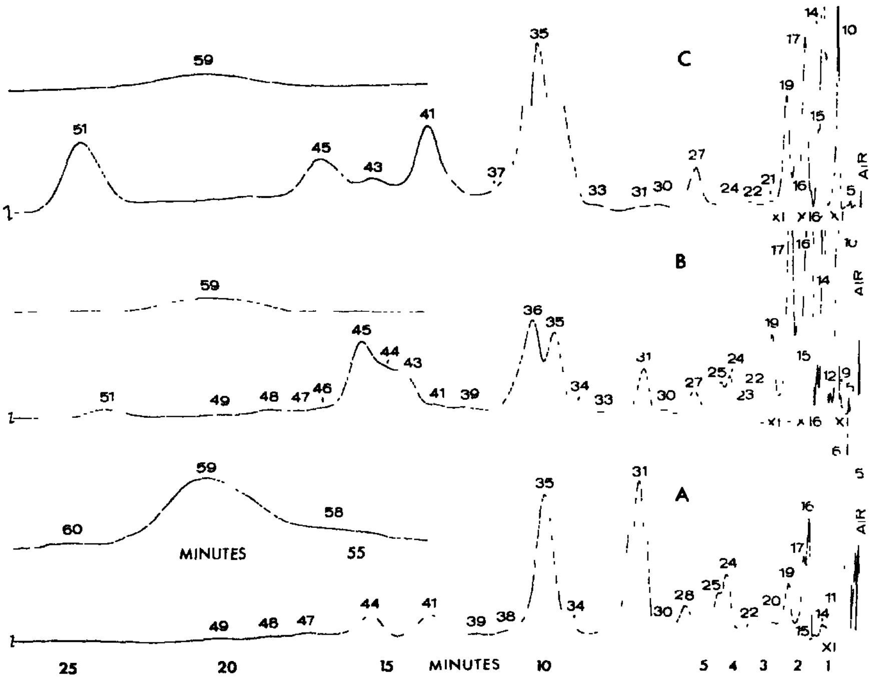

Fig. 1. Gas chromatographic patterns of the essential pulp-, rind- and leaf oils of *Poncirus trifoliata* L.

(A) pulp; (B) rind; (C) leaves.

Sample size: 1, 3·5 and 20 μl; Injection temperature: 210°; Column: 160°; Detector: 210°; Carrier gas: helium, 60 ml/min, 40 lb/in^2 pressure; Filament current: 200 mA; Leeds and Northrup recorder range: 1 mV; Chartspeed: 2·5 cm/min.

Intergeneric Hybrid Rind Oils

Rind oils of a number of selected *Poncirus* intergeneric hybrids with varieties of *Citrus* were obtained for use as possibly atypical essential oils and to lay the groundwork for future hybrid studies. Composite samples of ten fruits from each hybrid tree and from several trees of each *Citrus* parental clone were blended, distilled and stored as was done with the *Poncirus* selections.

GLC Operation Parameters

An Aerograph A-90-P Gaschromatograph with a thermal conductivity detector was used to determine the retention properties of the component of all oil samples in this study.

Of several columns tried, the best separation of the components was effected with a pyrex (150 cm) column with 6 mm i.d., packed with 60–80 mesh Chromosorb P, coated with 20% LAC 446. The other parameters are given in Fig. 1. The presence of the components was established by use of different columns, various sample sizes and changes in column temperature. The retention properties of the individual peaks were determined by com-

TABLE 2. THE ESSENTIAL RIND OILS OF VARIOUS *Poncirus trifoliata* L. SELECTIONS

Peak No.	Actual relative retention time range	Relative concentrations (%) of rind oil components of *Poncirus* selections									
		1*	2	3	4	5	6	7	8	9	10
10	1·00	0·70	0·96	0·52	0·53	0·77	0·78	0·66	0·80	0·64	0·95
12	1·27–1·40	0·03	0·12	0·04	0·04	0·03	0·08	0·05	0·05	0·06	0·09
13–14	1·55–1·70	24·01	27·48	34·79	27·72	31·34	36·13	32·30	34·03	37·10	25·28
15	1·86–2·03	3·97	3·35	3·36	2·03	2·06	1·99	2·80	3·06	3·82	3·98
16	2·12–2·31	47·39	41·14	35·53	52·37	49·38	32·84	41·40	29·95	31·35	44·54
17	2·40–2·60	13·34	11·96	13·39	8·31	9·06	11·46	11·83	15·35	15·89	14·68
19	3·02–3·50	1·33	1·49	2·01	0·93	0·91	0·32	1·57	2·47	2·25	0·55
20	0·37–0·40	0·24	0·73	0·24	0·49	0·45	1·10	0·32	0·22	0·26	1·65
21	0·41–0·43	0·33†		0·33			0·10	0·12	0·40	0·23	0·11
22	0·48–0·50	0·18	0·26	0·22	0·05	0·10	0·64	0·10	0·24	0·20	0·47
23–24	0·54–0·59	0·94	0·30	0·70	0·04	0·11	0·28	0·35	0·63†	0·53	0·18
25	0·63–0·68	0·23	0·26	0·46		0·07	0·23		0·69	0·43	0·44
26–27	0·70–0·76	0·39†	0·44	0·72	0·12	0·18	0·42	0·29	0·60	0·47	0·28
28	0·79–0·81		0·09		0·02	0·11					
29	0·85–0·90	0·09	0·19	0·20	0·02	0·11	0·37		0·15	0·11	0·37
31	1·00	0·26	0·62	0·72	0·24	0·23	0·19	0·26	0·69	0·47	0·14
33	1·12–1·21	0·08	0·30				0·27		0·17	0·06	0·11
34	1·25–1·30	0·06	0·32	0·16		0·08	0·32	0·06	0·25	0·18	
35	1·35–1·42	2·15	1·34	1·57	1·19	1·14	2·07	1·08	1·40	1·27	2·79
36	1·49–1·52	0·86	1·36	0·63	0·04	0·33	1·05	0·44	0·95	0·76	1·22
37	1·58–1·61	0·47	0·80	0·63	0·45	0·34		0·38	0·53	0·34	
38–39	1·77–1·82	0·31†	0·47	0·60	0·26	0·13	0·62	0·24	0·52	0·28	
40–41	1·86–1·96	0·24	0·44		0·23	0·21	0·87†	0·24	0·39	0·25	0·80
43	2·09–2·13	0·64	1·66	1·20	1·81	1·07		1·10	1·23	0·66	
44	2·17–2·20						1·76			0·45	0·55
45	2·30–2·37	0·84†	1·99	1·96	1·49	0·66	0·83	1·50	3·18	1·47	0·22
47	2·47–2·56	0·16	0·62		0·28			0·16	0·45		
48	2·56–2·72	0·08	0·96		0·24	0·23†	1·06	0·77		0·35	0·20
49	2·74–2·86						0·22	0·61	1·08		0·23
50	3·06–3·12	0·20				0·15	0·24				0·57
51	3·49–3·95	0·03	0·29			0·29	0·03	0·23	0·50	0·07	

* 1. Pomeroy; 2. Webber–Fawcett; 3. Christensen; 4. Rubidoux; 5. Barnes; 6. Taylor; 7. English; 8. Kryder 28-3; 9. Kryder 28-5; 10. Richmond.

† Unresolved peaks counted as one area.

paring their retention times with those of pure standards taken under identical conditions, and by addition of known quantities of standards to the sample. (Since this technique determines probable identity of the peak components, use of the standard compound name implies a component of the oil which has the same retention properties as the standard.) The area under the peaks was computed by the trapezoidal approximation method with a retention time increment of 6 sec.[9]

[9] G. B. THOMAS, JR., *Calculus and Analytic Geometry* (3rd Ed.). Addison-Wesley, Reading, Mass. (1962).

RESULTS AND DISCUSSION

Typical Poncirus *Essential Oils*

Table 1 shows the composition in per cent of the essential leaf, rind and pulp oils of *Poncirus trifoliata* L. These oils do not differ much qualitatively in the terpene and sesquiterpene fraction, but show some differences in the oxygenated fraction (Fig. 1). Quantitatively, however, wide variations exist. Significant is the large amount of linalool in the pulp (Fig. 1A); it appears in much smaller amounts in the rind and even less in the leaves. Nerol, nonanal, octanal, (+)-limonene, phellandrene and β-myrcene also exhibit quantitative differences (Table 1).

TABLE 3. THE ESSENTIAL RIND OILS IN TWO SELECTIONS OF *Poncirus*

Peak No.	Relative concentrations (%) of rind oil components in fruits from north and south sides of selected trees: Pomeroy						Rubidoux					
	1N	2N	3N	1S	2S	3S	4N	5N	6N	4S	5S	6S
10	1·86	1·04	1·84	1·83	1·62	1·80	0·75	1·31	0·60	0·88	1·31	0·99
12	0·22	0·12	0·17	0·18	0·16	0·18	0·04	0·06	0·03	0·05	0·07	0·05
14	32·23	27·56	27·07	28·31	25·86	30·18	26·52	24·70	24·52	25·23	28·55	27·76
15	5·33	4·28	4 61	4·25	4·23	4·24	3·54	3·69	3·08	3·51	3·25	3·16
16	36·73	44·19	44·34	45·76	45·01	42·44	51·37	54·36	51·09	52·98	51·82	52·76
17	15·61	13·54	12·55	10·61	12·68	13·68	12·79	11·19	11·30	9·87	10·28	11·58
19	0·46	0·21	0·46	0·44	0·37	0·47	0·21	0·31	0·24	0·25	0·29	0·26
20	0·19	0·27	0·20	0·25	0·19	0·21	0·21	0·39	0·22	0·27	0 34	0·23
22	0·18	0·12	0·12	0·12	0·06	0·12	0·26	0·55	0·35	0·33	0·20	0·23
27	0·17	0·09	0·12	0·10	0·06	0·09	0·13	0·06	0·26	0·25	0·07	0 07
31	0·14	0·06	0·17	0·14	0·06	0·12						
33	0·36	0·13	0·29	0·18	0·17	0·24	0·10	0·13	0·13	0·16	0·07	0·10
35	3·45	6·09	4·20	4·84	6·09	3·48	1·74	1·47	3·34	2·58	2·30	1·84
36–38	1·08	1·10	1·50	1·18	1·37	1·18	0·44	0·29	0·93	0·85	0·47	0·33
40–41	0·31	0·09	0·37	0·18	0·37	0·24	0·21	0·12	0·48	0·33	0·05	0·01
42–43	0·53	0·45	0·58	0·53	0·71	0·47	0·21	0 05	0·48	0·41	0·05	0·03
45	0·56	0·39	0·63	0·65	0·62	0·41	1·22	1·10	1·80	1·54	0·87	0·56
48	0·58	0·30	0·78	0·47	0·37	0·47	0·26	0·29	1·16	0·52	0·10	0 07

All selections of trifoliate orange can easily be recognized as trifoliate by their characteristic patterns (Table 2). When observing "fingerprints" of the oils, however, it must be borne in mind that the importance of these patterns does not rest upon an individual component or peak, but upon all of them together, especially in their relationships to each other. Only one or very few components are necessary for taxonomic differentiation, as long as these are taxonomically sound and consistent. The "fingerprint" characteristic for *Poncirus* consists of the peak groups 10, 35–38 and 41–45, which are quite different from other taxa tested.

One trifoliate selection tested, which does not exhibit this pattern, is the small-flowered Taylor trifoliate.

Natural Variation of Poncirus *Oils*

Table 3 shows the variability of the essential rind oils within two selections, Pomeroy (large-flowered) and Rubidoux (small-flowered), with respect to location of the fruits on the

TABLE 4. VARIATION BETWEEN TWO SELECTIONS OF *Poncirus* AT THREE HARVEST PERIODS, WITH RESPECT TO MEAN RETENTION PROPERTIES OF RIND OIL COMPONENTS

Peak No.	Variation of mean relative retention times of rind oil components		Mean relative retention volumes of rind oil components															
			13 September					5 October					20 October					
			Pomeroy			Rubidoux		Pomeroy			Rubidoux		Pomeroy			Rubidoux		
	Mean	C.V. %	Mean	C.V. %	F	Mean	C.V. %	Mean	C.V. %	F	Mean	C.V. %	Mean	C.V. %	F	Mean	C.V. %
10	1·00	—†	1·67	19·2	*	0·97	30·0	1·18	23·1	*	0·70	21·5	1·41	21·9	NS	0·96	21·4
12	1·34	1·9	0·17	19·1	*	0·05	63·2	0·11	21·5	*	0·05	56·4	0·11	24·1	**	0·05	20·0
14	1·66	1·6	28·54	8·1	NS	26·21	6·4	26·71	6·1	NS	25·19	4·3	27·21	5·7	*	24·18	5·8
15	1·95	2·1	4·43	9·7	**	3·37	1·2	4·78	11·4	*	3·45	7·9	4·66	8·4	*	3·91	6·8
16	2·24	2·5	43·08	7·7	**	52·40	2·6	44·34	8·3	*	53·26	2·9	43·00	5·5	**	53·52	3·7
17	2·49	2·7	13·11	12·5	**	11·17	9·2	13·80	10·4	NS	12·32	3·6	14·25	10·1	*	12·21	4·1
19	3·36	3·8	0·40	25·1	NS	0·26	14·9	0·48	14·1	NS	0·41	16·2	0·48	16·4	NS	0·48	31·6
20	0·39	6·6	0·22	15·7	NS	0·28	26·3	0·14	20·1	*	0·08	35·3	0·11	32·8	NS	0·10	34·6
22	0·46	2·0	0·12	31·2	*	0·32	39·5	0·15	37·1	NS	0·14	50·5	0·18	18·4	NS	0·15	58·5
27	0·76	1·5	0·11	34·0	NS	0·14	66·2	0·11	38·5	NS	0·11	41·6	0·09	41·6	NS	0·11	30·2
31	1·00	—‡	0·12	38·2	—	tr.	—	0·15	32·0	—	tr.	—	0·13	57·5	—	tr.	—
33	1·13	1·4	0·23	37·1	*	0·12	26·3	0·30	43·8	*	0·10	64·8	0·20	26·0	**	0·07	32·0
35	1·43	2·5	4·69	25·6	*	2·21	30·9	4·80	24·0	*	2·19	31·5	5·68	9·5	**	2·26	31·6
36–38	1·62	2·2	1·24	13·3	*	0·55	49·4	1·17	24·1	*	0·50	36·2	1·39	16·4	*	0·57	43·2
40–41	1·90	3·7	0·26	43·0	NS	0·20	89·6	0·48	23·0	*	0·19	31·6	tr.	—	—	0·09	65·8
42–43	2·02	2·5	0·55	17·0	*	0·21	74·2	0·20	70·0	NS	0·17	55·8	0·50	33·2	NS	0·33	47·7
45	2·35	1·5	0·54	8·7	*	1·18	38·0	0·60	28·6	NS	0·93	26·0	0·51	15·4	*	0·94	35·4
48	2·70	2·4	0·50	33·9	NS	0·40	101·4	0·38	40·3	*	0·15	52·1	tr.	—	—	tr.	—

C.V. %—Coefficient of Variation, the ratio of the standard deviation to the mean.
F—Significance of difference. Confidence: ** = 99 %, * = 95 %, NS = No significance.
† Internal standard for terpene retention time calculations (α-pinene).
‡ Internal standard for sesquiterpene retention time calculations (linalool).

tree and type of tree. In each selection, fruits from the north and the south side were harvested separately in order to study possible influences of light and temperature upon the composition of the terpenes. No consistent differences were found in fruits from the north and south sides of the trees, but differences between the two strains were immediately apparent.

It was possible to study the individual components independently of one another. However, a correlation existed among the components, probably due to conversion of precursors, isomerism, interconversion and degradation. The study was continued until maturity, with later harvests from the same trees.

In the tests made the physiological ages of the different selections were not comparable. Of these selections Pomeroy was chosen as representative of the large-flowered group and Rubidoux of the small-flowered group. In addition to the specific morphological characters there were also variations in growth characteristics, such as branching habit, time of flowering, time of deciduousness, leaf emergence, fruit maturation and fruit drop.

In Table 4 one can observe the statistical variations which existed within and between the Pomeroy and Rubidoux selections on three different dates of harvest: 13 September, 5 October and 20 October 1965. The mean relative per cent composition expressed represents six samples of each selection, while the mean retention times represent thirty-six chromatographic analyses. Rind oil GLC determinations for these fruits have shown no significant differences between the strains as expressed in prominent chromatogram features except peaks 12, 15, 16, 31, 33, 35 and 36–38. Components which could be significantly used to differentiate Pomeroy and Rubidoux in this study were (+)-limonene (16) and nonanol (35). Other peaks could have been used except for their natural variability within strains. It has been shown that the coefficient of variability was high when the prominent feature or peak was the location of several unresolved components with similar retention properties.

Studies on hybrids from crosses of *Poncirus* with Red lime (*Citrus aurantifolia*), Ruby orange (*C. sinensis*) and Clementine mandarin (*C. reticulata*) have shown that the chromatographic "fingerprints" of the hybrids were not intermediate, but varied greatly among the progeny. The components of the essential oils behaved somewhat independently of one another and certain peaks therefore were intermediate, while others resembled one or the other parent. Hybrid patterns, however, were recognizable. The greatest difficulty encountered was the influence of maturity factors upon the individual components of the essential oils. This was especially important in crosses where one parent might have been early-maturing and the other late. The maturity of their hybrids often ranged between the maturity dates of the two parents. In such cases it was difficult to compare the hybrids with each other if they were not of the same physiological age. Chromatographic features which have shown promise in the differentiation of such hybrids included 10, 15, 16, 17, 24, 25, 27, 35, 36 and 41–45. The natural fluctuation of component quantity, however, must be well-understood in order not to be interpreted as a differentiating characteristic for taxonomic classification. It is important, then, to explore the most appropriate time of harvesting in order to obtain data for comparative purposes.

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