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EFFECT OF OLEOCELLOSIS, DESICCATION, AND FUNGAL INFECTION UPON THE TERPENES OF INDIVIDUAL OIL GLANDS IN *CITRUS LATIPES*

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Key Word Index—*Citrus latipes*; Rutaceae; terpenes; individual oil glands.

Abstract—Mono- and sesqui-terpenes of individual oil glands of *Citrus latipes* fruits were analyzed for their homogeneity. The effect of oleocellosis, desiccation, *Penicillium* and *Phytophthora* infection upon the individual terpene components was investigated and expressed in a discriminant analysis and in canonical variables. Each oil gland contained the entire spectrum of terpenes specific for each species, and the biggest difference in affected glands was due to *Penicillium* infection.

INTRODUCTION

STUDIES on *Poncirus*, a genus closely related to *Citrus*, have shown that differences exist in the ratios of the essential terpene components of fruit harvested on the north side of trees compared to those harvested on the south side of the same trees.[1] Since the synthesis of mono- and sesqui-terpenes and the ratios of their individual components depend upon light intensity, photosynthesis and the heat pattern of the fruit surface, such differences are expectable.

The availability of a micromethod enabling us to analyze the terpene content of one single oil gland offered the opportunity for investigating whether a single oil gland contained either the entire spectrum of terpenes typical for a species, or only a part of the mono- and sesqui-terpenes which, upon mixing with the content of the other oil glands resulted in the specific species pattern. This method was also useful for determining whether there were any terpene differences in individual oil glands due to their location on the rind.

This single oil gland analysis also permitted us to investigate the effects of oleocellosis, desiccation, and of infections of *Penicillium digitatum* Sacc., and *Phytophthora citrophthora* (Sm. and Sm.) Leon, upon the individual terpene components of single oil glands.

Citrus oils are toxic to their own fruit tissues. Oleocellosis, which is due to action of oil liberation from the oil glands of the rind results in rindstaining. This causes economic loss. The inquiry into the stability of rind oils in the above interactions might, therefore, bear upon the shelf life of citrus fruits, and is thus of economic importance. *Citrus latipes* (Swing.) Tan. was chosen for this study because its fruits possess large oil glands.

RESULTS AND DISCUSSION

It was found that each oil gland tested contained the entire spectrum of terpenes specific for the species, and that in this study the location of the oil gland on the fruit had no influence upon its mono- and sesqui-terpene pattern.

The oil glands of several individual healthy fruits were compared with each other to find

[1] SCORA, R. W. and BITTERS, W. P. (1967) *Lloydia* **30**, 182.

fruit-to-fruit variation. A one way analysis of variance, and F test were performed for each of the 20 components tested (5 or 6 glands were taken from each fruit.) Table 1 shows the mean percentages for each fruit on each component peak. α-Pinene, sabinene, β-myrcene, terpinolene, and furfural show significant differences between fruits at the 5% level; peaks 8 and 9 (unknowns) show them at the 1% level. Although significant, the differences in means per fruit are small.

TABLE 1. ANALYSIS OF *Citrus latipes* LEAF OILS

Peak No.		Healthy glands, mean % Fruit 1	2	3	4		s.d.	Type of gland, mean % Healthy	Oleo	Desi	Pen	Phyto	
1	α-Pinene‡	5·96	4·04	4·24	5·42	*	1·16	4·94	3·51	4·32	6·66	3·39	**†
2	β-Pinene‡	2·37	1·71	1·79	2·35		0·53	2·07	1·49	2·00	1·82	1·40	**
3	Sabinene	0·71	0·46	0·50	0·54	*	0·13	0·55	0·35	0·41	0·39	0·34	**
4	β-Myrcene‡	6·79	4·31	4·88	5·07	*	1·11	5·26	3·40	4·41	4·26	3·60	**
5	*d*-Limonene‡	53·09	64·13	61·02	53·82		8·26	57·81	70·51	60·85	59·60	68·37	**
6	γ-Terpinene‡	18·18	13·78	15·19	16·05		2·57	15·81	11·98	15·51	15·63	12·67	**
7	Terpinolene‡	4·49	4·83	3·87	9·77	*	3·29	5·93	4·35	6·97	6·16	3·31	
8	Unknown	0·05	Tr	0·13	0·01	**	0·06	0·04	Tr	0·02	Tr	0·05	
9	Unknown	0·27	0·11	0·29	0·04	**	0·10	0·17	0·02	Tr	0·01	0·02	**
10	Furfural	0·11	0·12	0·11	0·17	*	0·04	0·13	0·16	0·43	0·19	0·46	**
11	α-Copaene	0·05	0·07	0·10	0·08		0·04	0·08	0·03	0·09	0·07	0·04	*
12	Linalool	0·14	0·17	0·23	0·23		0·09	0·19	0·03	0·06	0·10	0·03	**
13	β-Elemene	0·01	0·03	Tr	Tr		0·02	0·01	Tr	Tr	0·11	Tr	**
14	Caryophyllene‡	1·44	1·03	1·30	1·38		0·37	1·29	0·93	0·96	0·83	1·13	*
15	Terpinen-4-ol	0·24	0·21	0·24	0·20		0·10	0·22	0·15	0·09	0·64	0·13	**
16	Cadinene isomer	0·25	0·24	0·25	0·16		0·11	0·22	0·16	0·17	0·19	0·18	
17	α-Terpineol‡	2·60	2·06	1·91	2·16		0·53	2·18	1·35	1·28	1·58	1·82	**
18	Citronellol	0·86	0·50	0·65	0·61		0·24	0·66	0·25	0·42	0·30	0·67	**
19	Geranyl acetate	0·46	0·30	0·43	0·24		0·17	0·35	0·09	0·14	0·30	0·23	**
20	Nerolidol‡	1·94	1·90	2·87	1·71		0·68	2·09	1·25	1·88	1·19	2·15	*

† One asterisks indicates a 5% significant difference between means, two asterisks indicate a 1% significant difference.

‡ IR identification.

The oil patterns of the healthy glands were then compared with those of the desiccated and oleocellosis stained glands, and also with those affected with *Penicillium* and *Phytophthora*.

Some terpene changes were immediately visible, especially the increase in β-elemene and terpinen-4-ol in the *Penicillium*-affected oil glands when compared to the healthy glands. Among the terpene components found in *C. latipes*, a few are also found in the filamentous fungus *Ceratocystis variospora* (Davidson) Moreau, namely, linalool, citronellol, and geranyl acetate.[2] These components might also be produced by the fungus *Penicillium*. However, the values of these above components are lower in the *Penicillium*-infected oil mixture than in the mixture in healthy glands, and therefore a contamination with oil components proper to *Penicillium* seems unlikely. Furfural showed a visible increase in the desiccated and *Phytophthora*-infected oil, resulting, in the latter case perhaps, from the decomposition of sugar by the fungus.

Table 1 shows the mean percentages obtained from the one way analysis of variance and F test for the five types of glands (healthy, desiccated and oleocellosis-, *Penicillium*-, or *Phytophthora*-affected) on each peak. These types were significantly different for all the components except terpinolene, the unknown peak 8 and a cadinene isomer. We also analyzed these data on a stepwise discriminant program[3,4] which selected the peaks that

[2] COLLINS, R. P. and HALIM, A. F. (1970) *Lloydia* **33**, 481.

[3] DIXON, W. J. (1967) *Biomedical Computer Programs*, pp. 214a–214t, Univ. of Calif. Press, Berkeley.

[4] SOKAL, R. R. and ROHLF, F. J. (1969) *Biometry the Principles and Practice of Statistics in Biological Research*, pp. 488–490, Freeman, San Francisco.

contributed most significantly to the discrimination between the five types of glands. The peaks selected were 1, 2, 4, 5, 10, 12, 14, 15 and 17. Peaks not selected showed no type difference or duplicated information contained in the chosen peaks. The assignment of individual glands to the correct gland type on the basis of discriminant functions using the nine selected peaks was perfect in every case except one. Here, an oleocellosis gland was classified as a *Phytophthora* affected gland.

In addition to the discriminant analysis, a canonical analysis[5] was performed. The canonical variables are linear transformations of the same nine selected peaks, which provide optimum separation of gland types. They were calculated in decreasing order of importance so that just the first two provided good separation of types. Figure 1 shows these first two canonical variables. The *Penicillium*-infected glands were widely separated by the first canonical variable; the other groups of glands were separated to a lesser degree. The healthy glands separated then on the second canonical variable.

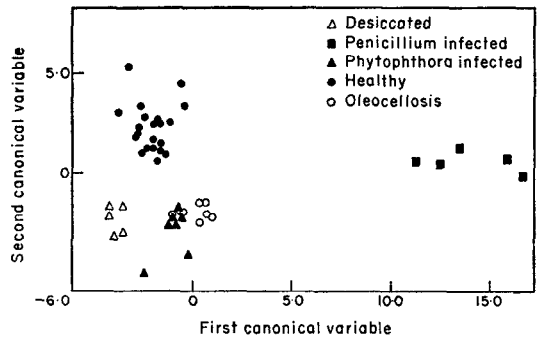

FIG. 1. PLOT OF FIRST AND SECOND CANONICAL VARIABLES FOR NINE SELECTED TERPENE PEAKS.

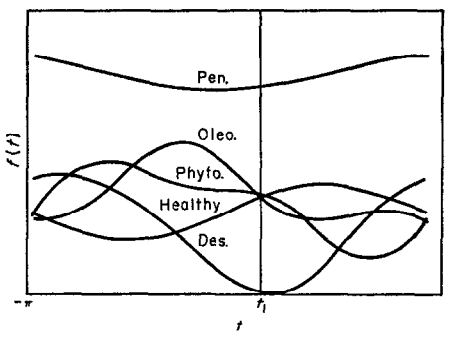

FIG. 2. FULL CANONICAL ANALYSIS OF NINE SELECTED TERPENE PEAKS.

Figure 2 shows a curve for each of the above gland types based on all nine canonical variables. This function, using several variables, served as a graphical aid for presenting the types of glands. Each of the five types of oil glands turned out to be a very distinct entity. *Penicillium*-infected oil was found to be the most different one; healthy, oleocellosis and *Phytophthora*-infected oils ranged closest together, as indicated by the convergence of the curves at t_1.

In summary, it can be said that in this experiment of analyzing individual oil glands of *Citrus latipes*, each oil gland contained the entire spectrum of terpenes specific for the species. There was no variation of terpene spectra due to the location of the individual oil glands on the fruit; there were, however, fruit-to-fruit differences in the terpene spectra of healthy fruits. In affected glands the biggest difference was due to *Penicillium* infection.

EXPERIMENTAL

Plant material. All fruits were personally harvested from tree CRC 3052 in our Citrus Varietal Collection in field 19C Row 10 Tree 8 at UCR, Riverside, Ca. 6 glands were taken for analysis from each of 4 healthy fruits. 6 glands were taken from each of 2 Oleocellosis-affected fruits; 5 glands from 1 desiccated fruit; 5 glands from 1 *Penicillium*-infected fruit and 5 and 2 glands from 2 *Phytophthora*-infected fruits.

Determination of the content of individual oil glands. Mature fruits of *Citrus latipes* were used. The oils were extracted with a Hamilton syringe which was modified in the needle tip and combined with a low

[5] SEAL, H. L. (1964) *Multivariate Statistical Analysis for Biologists*, pp. 124–144, Wiley, New York.

vacuum pump. Thus, the whole content within one oil gland membrane was taken up. The oil was injected into a Varian 1520 GLC with a FID. Two 305 × 0·64 cm matched stainless steel columns packed with Chromosorb W which was coated with 20% diethylglycol adipate with pentaerythritol DMCS were used for analysis. Injection temperatures were 200°. Helium and hydrogen flow were 24 ml/min; air flow was 200 ml/min. The column temperature was programmed non-linearly from 50 to 182° in a 2·5 hr run.

Identification of the individual oil components. Hand-pressed oil of the healthy fruits was collected. This was analyzed on a larger column system but with a thermoconductivity detector, in order to catch the individual oil components in capillary tubes at the detector outlet. These were identified with IR, and the small ones, too small to catch, by augmentation with our reference samples.

The function[6] used for the separation of oil types by 9 canonical variables (Fig. 2) was: $f(t) = s_1\sqrt{2} + x_2 \sin t + x_3 \cos t + x_4 \sin 2t + x_5 \cos 2t + x_6 \sin 3t + x_7 \cos 3t + x_8 \sin 4t + s_9 \cos 4t$, where X_1, $x_2, \ldots, x_9$ are the canonical variables and t varies from $-\pi$ to π.

[6] ANDREWS, D. F. (1972) *Biometrics* **28,** 125.