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Relationship of Rootstock to Leaf and Juice Lipids in Citrus[1]

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Abstract. **Major leaf alkanes (C29-C33) of 2 scions on 10 rootstocks of citrus were examined by gas chromatography. A small but definite effect of the rootstock on the alkane profiles of the scions was observed. The effect of rootstock on alkane patterns in juice sacs was very small. Rootstock affected the fatty acid patterns of total and neutral lipids as well as of triglycerides and sterol esters.**

Information on the chemical composition of the epicuticular wax layer of citrus leaves is important if efficient use of sprays of growth regulators, leaf nutrients, pesticides, and antitranspirants is to be attained. Long-chain hydrocarbons (alkanes), the major component of this wax layer, vary among the citrus species both quantitatively (1) and qualitatively (1, 11). Of the many classes of compounds present in waxes, the alkanes are the least wettable. Since water repellency of plant surfaces affects chemical deposition, leaf waxes with high contents of alkanes and/or different hydrocarbon compositions would be expected to affect the deposition of chemicals and their absorption by the leaf. Another epicuticular wax surrounds the juice vesicles, and holds them together. The quality of citrus sections is related to the composition of this wax layer of which alkanes are a major component (12). Fatty acids are major lipid constituents found within the juice vesicle.

The majority of citrus trees grown today are budded on rootstocks, and there are numerous examples of scion-rootstock interactions (2, 3, 4, 5, 6, 13). Our previous studies (9, 10, 11) were conducted on seedling trees to avoid any rootstock effects on the hydrocarbon, fatty acid, and sterol profiles of the scions. The present experiment was conducted to study the effects of rootstock on leaf and juice lipids.

Materials and Methods

The leaves and fruit used in these studies were from Whitmore Foundation Farm (U. S. Horticultural Research Laboratory, Orlando, Florida). Leaves were from trees consisting of 2 scions, 'Orlando' tangelo (*Citrus paradisi* Macf. x *C. reticulata* Blanco) and 'Owari' satsuma (*C. reticulata*), on 10 rootstocks; Rusk (RSK) citrange [*C. sinensis* (L.) Osbeck] x [*Poncirus trifoliata* (L.) Raf.], Troyer (TROY) and Carrizo (CAR) citranges (*P. trifoliata* x *C. sinensis*), Large Flower trifoliate orange (LFTO) (*P. trifoliata*), Seville sour orange (SO) (*C. aurantium* L.), Cleopatra mandarin (CLEO) (*C. reticulata*), Sanquine Grosse Ronde sweet orange (SANG) (*C. sinensis*), Orlando tangelo (ORL) (*C. paradisi* x *C. reticulata*), Estes rough lemon (RL) [*C. limon* (L.) Burm. f.], and Milam (MIL) (rough lemon hybrid?).

The trees, planted on Astatula fine sand in 1965, were spaced 4.5 x 5.4 m in a randomized block with 3-tree plots. The trees had regular and uniform horticultural attention and were vigorous and fruitful. Six replicates of each scion-rootstock combination were taken from these 3-tree plots. A sample consisted of 20 to 30 mature leaves picked from each of the 3 trees on both September 8 and October 13, 1976.

The epicuticular wax was removed from the leaves by dipping them for 3 min in 200 ml chloroform. The alkanes were isolated from this extract by thin-layer chromatography (11). Fruit for studies on the alkanes and fatty acids of juice were obtained on December 14, 1976, from 18 of the same trees ('Orlando' on 6 rootstocks) used in the leaf alkane study. A sample consisted of 12 fruit picked from the south side of the tree. The fruit were cut in half, and the intact juice sacs were carefully separated from core, peel, seeds, and carpellary membrane with the aid of a citrus spoon. The lipids were extracted with Folch's reagent from a Celite pad (8) and the major portion of the lipid extracts fractionated into neutral lipids (NL), glycolipids (GL), and polar lipids (PL) (9). Triglycerides (TG) and sterol esters (SE) were isolated from a portion of the NL fractions by thin-layer chromatography (9). Methyl esters of the fatty acids (FAMEs) from total lipids (TL), NL, GL, and PL lipids, as well as from TG and SE, were prepared and purified along with the alkanes by thin-layer chromatography (9) and stored in hexane for gas-liquid chromatography (9). All alkanes and FAMEs were analyzed on a 182 cm x 0.6 mm glass 3% SP1000 on 100/120 Gas Chrom Q gas-liquid chromatographic column that was run isothermally at 160°C for FAMEs (9), and temperature programmed for juice sac (10) and leaf (11) alkanes. Duplicate gas-liquid chromatographic analyses were run on each leaf alkane sample. A single gas-liquid chromatographic analysis was run on each of the six juice-sac alkane samples obtained (3 from TL, 3 from NL) from each scion-rootstock combination. Duplicate gas-liquid chromatographic analyses were run on the TL, NL, TG, and SE FAME samples, whereas a single analysis was run on each of the GL and PL FAME samples.

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Results and Discussion

Relative percentages for the five C29-C-33 leaf hydrocarbons of mature leaves (11) from 'Orlando' tangelo and 'Owari' satsuma scions on 10 rootstocks are shown in Table 1. Each value represents the mean of 6 replications. The coefficient of variation (CV) for several mean ranges (MR) showed the following: MR less than 10% (CV = 2–45%); MR 10 to 50% (CV = 2–22%); MR above 50% (CV $<$ 6%). Each of the 'Orlando' scions on 9 rootstocks were statistically compared to the 'Orlando' on ORL rootstock by the t-test for 2 means. The profiles of the 10 scion-rootstock hydrocarbons were noteably similar. However, 14 statistically different means were recorded for the C_{29}, C_{30}, C_{31} and C_{33} hydrocarbons (Table 1). Of these 14 different means RSK, TROY and LFTO showed the greatest differences from the 'Orlando'-ORL standard.

The 'Owari' on CLEO was chosen as the standard for comparison of the 'Owari' scion-rootstock effects because an 'Owari' scion-'Owari' rootstock combination was not available. 'Owari' and 'Cleopatra' are mandarins that possess similar and distinct leaf alkane profiles. In general the 9 scion-rootstock profiles were similar to the 'Owari'-CLEO profile. About 8 rootstocks were higher in C_{29}, C_{30}, and C_{32} values, and lower in C_{31} values when compared to 'Owari' on CLEO. Sixteen statistically different means were recorded for C_{29}, C_{30}, C_{31}, C_{32} and C_{33} (Table 1). The 3 citranges, RSK, TROY and CAR, showed the most difference from the control.

In order to detect the influence of rootstock on juice-sac alkanes, we examined the waxes from juice sacs of 'Orlando' on 6 different rootstocks (Table 2). To simplify this study, only major alkanes were examined (C20-C27). Iso and anteiso branched alkanes of similar chain length were combined. The means were compared to the 'Orlando'-ORL mean by the t-test. Minimal but significant differences between alkane profiles from these rootstocks were observed in branched and linear C_{22}, branched and linear C23 and linear C27 compounds. The differences between 3 seedling tangelo scions, 'Orlando', 'Minneola', and 'Seminole' reported in a previous study (7), far exceeded the respective differences between the 6 'Orlando' scion-rootstock combinations. Thus, the influence of rootstock on the alkane profile of the juice sacs of 'Orlando' tangelo may be regarded as minimal. Preliminary analysis of the FAMEs of the neutral lipid, glycolipid and phospholipid fractions showed that the majority of fatty acid changes occurred in the neutral lipid fraction. Therefore, fatty acid analysis were conducted only on total lipids, neutral lipids, and two major lipids of the neutral lipid fraction, namely, triglycerides and sterol esters. The means of the 3 major fatty acids (C18:1, C18:2, C18:3) with the greatest change or difference are shown in Table 3.

Of the 3 major acids in TL, NL, TG, and SE, C18:1 showed the greatest range of values between rootstocks with decreasing ranges observed for C18:2 and C18:3 (Table 3). The C18:1 $>$ C18:2 $>$ C18:3 sequence of ranges was not observed in GL and PL. This is further evidence that the greatest influence a rootstock has on the fatty acid profile of citrus is apparent in the NL. The sequence in which the rootstocks are presented in Table 3 follows a trend in relative percent values for these 3 fatty acids present in TL. This trend is from low values (RSK, 36.0%) to high (MIL, 40.0) for C18:1 and from high (RSK 18:2, 27.2%) to low (MIL 18:2, 24.6%) for both C18:2 and C18:3. These same trends are present also in NL, TG, and SE. The fatty acids of the 5 rootstocks were compared to the 'Orlando' on ORL fatty acid profiles by the t-test. With the exception of MIL, various fatty acid trends were apparent between the reference, 'Orlando' on ORL, and the other four rootstocks. As an example, decreasing 18:1 values (in TL and NL) were observed for the rootstock sequence SANG $>$ CLEO $>$ SO $>$ RSK. Other trends and fatty acid differences were also noted (Table 3). The following number of significant differences in fatty acid means from the 'Orlando' reference were shown: Rusk 10, Sour Orange 8, Cleopatra 8, Sanquine Sweet Orange 6 and Milam 3.

Table 1. Distribution of hydrocarbons in leaves of 'Orlando' and 'Owari' scions on 10 rootstocks.

	Distribution of hydrocarbons (%)									
	'Orlando' scion					'Owari' scion				
Rootstock[z]	C29	C30	C31	C32	C33	C29	C30	C31	C32	C33
RSK	8.9*[y]	4.6*	50.0	8.7	27.8*	20.4*	4.2*	56.6*	5.0	13.8
TROY	8.5*	4.5	49.8	9.1	28.1*	18.0*	3.7*	59.6*	5.1*	13.6
CAR	7.3	3.5	51.0	8.6	29.6	16.4	3.9*	60.9*	4.8*	14.0
LFTO	8.3*	3.6	52.6*	8.2	27.3	16.7	3.7	64.3	3.6	11.7
SO	6.7	2.9	52.5*	8.1	29.8	17.1	3.1	61.7	4.3	13.8
SANG	7.0	3.2	51.2	8.6	30.0	19.5*	3.3	62.9	3.7	10.6*
RL	7.8	3.8	51.2	8.3	28.1*	14.4	3.6	64.1	4.5	13.4
MIL	6.6*	3.0	52.0	8.5	30.0	17.7*	3.7*	62.3*	4.2*	12.1
CLEO	7.4	3.4	51.9*	8.7	28.6*	*15.1*	*3.1*	*64.4*	*3.7*	*13.7*
ORL	*7.3*	*3.5*	*50.4*	*8.7*	*30.1*	16.5	3.0	63.0	4.6	12.9

[z]See text for listing of rootstocks.

[y]Values in each column with an asterisk are significantly different at the 5% level from the reference value (italicized).

Table 2. Distribution of hydrocarbons in juice of 'Orlando' scion on 6 rootstocks.

	Distribution of hydrocarbon[z] (%)													
Rootstock	L20	L21	Br22	L22	Br23	L23	Br24	L24	Br25	L25	Br26	L26	Br27	L27
RSK	0.3	1.2	0.5	2.7	17.7*	15.2	12.3	5.4	18.2	10.3	6.7	1.4	5.6	2.5
SO	0.3	0.5	0.4	2.5	18.7	15.1*	11.7	5.2	18.8	10.4	6.8	1.7	5.8	2.1
SANG	0.3	0.7	0.4	2.4	18.9	14.6	12.0	4.9	19.4	10.8	6.6	1.5	5.3	2.2
MIL	0.3	1.1	0.5	3.0	19.0	15.0	12.0	4.6	18.9	10.3	5.8	1.7	4.8	3.0
CLEO	0.2	1.3	0.7*[y]	3.3*	18.0	15.2	12.2	5.2	19.0	10.2	7.1	1.4	4.6	1.6*
ORL	*0.3*	*0.7*	*0.4*	*2.4*	*18.5*	*14.4*	*12.3*	*5.0*	*20.3*	*10.4*	*6.7*	*1.3*	*4.7*	*2.6*

[z]L and Br are linear and combined branched (iso and anteiso) hydrocarbons respectively.

[y]Values in each column with an asterisk are significantly different at the 5% level from the reference values (italicized).

Table 3. Values for 3 of the 6 major fatty acids in juice lipids of 'Orlando' scion on 6 rootstocks.

	Distribution of fatty acids (%)											
	Total lipid			Neutral lipid			Triglycerides			Sterol esters		
Rootstock	18:1	18:2	18:3	18:1	18:2	18:3	18:1	18:2	18:3	18:1	18:2	18:3
RSK	36.0*[z]	27.2*	11.7*	36.5*	29.6*	16.1*	44.6*	24.2*	13.3*	19.7*	52.3	19.1
SO	36.5*	27.1*	10.5	38.8*	29.7*	12.1	42.8*	25.7*	11.9	20.4*	53.6*	18.5
CLEO	37.6*	25.7*	11.1	39.9*	27.2*	13.9*	47.5	22.1	12.6	19.4*	53.1*	19.9*
SANG	37.6*	26.0*	11.2	40.7*	26.1	12.9	47.6	23.4*	10.7	20.2*	51.4	20.7*
ORL	*38.9*	*25.1*	*10.7*	*42.4*	*25.7*	*12.8*	*48.7*	*21.8*	*11.8*	*22.9*	*51.1*	*17.8*
MIL	40.0	24.6*	10.6	42.9	24.5*	12.2*	48.6	21.3	12.6	24.2	50.1	17.8

[z] Values in each column with an asterisk are significantly different at the 5% level from 'Orlando' on ORL values (italicized).

Conclusion

Rootstock type does not appear to be a major factor in determining the alkane pattern of the epicuticular wax of citrus leaves nor does it appear to influence the alkane pattern of the juice vesicular wax. The alkane profiles in both tissue studies were characteristic of the scion (11). The 6 rootstocks studies apparently had a definite influence on the juice fatty acid profile of the scions. Hutchison and Hearn (5) collected yield and juice quality data in 1976 from the trees used by us in the same year. 'Orlando' on 'Rusk' had the highest yield and the fruit had the highest total soluble solids and acid percentage. 'Milam' showed low yields of fruit and soluble solids (5). The data in Table 3 show noticeable differences between 'Rusk' and 'Milam', and might indicate possible relationships between fatty acid percentages and fruit quality (yield, soluble solids, acid).

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