THE PROXIMATE CHEMICAL CONSTITUENTS OF CITRUS WOODS, WITH SPECIAL REFERENCE TO LIGNIN[1]

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(WITH THREE FIGURES)

Introduction

Valencia orange fruit, which matures during the summer in California, is subject to a disorder recognizable especially when the fruit remains on the trees for some time after maturation. In its early stages, this disorder is characterized by a reduction in the amount of available juice in the vesicles in the proximal end of the fruit due to the gelation of the sap. Later there is an increase in the thickness of the walls of the cells composing the interior of the juice vesicle (fig. 1). This change is accompanied by gas formation and by changes in cell wall constitution. When treated with phloroglucinol and hydrochloric acid, the cell walls give a brilliant red lignin reaction (**16**) not shown by normal cell walls. This disorder is called "granulation" (**1**).

Sixteen constitutents known to be present in the orange fruit were tested with phloroglucinol and HCl. Two of these constitutents, crude orange oil and citral, formed transitory cerise colors. In the juice vesicle the cerise color remained several hours. E. C. CROCKER has shown that yellow to red colors develop with certain compounds containing aldehydes. We have confirmed his tests and concur with the view that coniferal aldehyde or hadromal probably are responsible for phloroglucinol-HCl color reaction in wood. Although the cell walls of granulated juice vesicles gave a positive phloroglucinol-HCl reaction for lignin, the reaction might be due to some other compound. Isolation of lignin from juice vesicles is in progress.

In this connection it was of interest to know whether there might be a tendency in various citrus species, varieties, and rootstock combinations toward greater lignification in the tree itself. It has long been recognized by DR. H. J. WEBBER and others that fruit is affected by the type of rootstock upon which the scions are budded or grafted and that certain rootstock and scion combinations are incompatible. Thus far-reaching changes might be expected to take place in the scions. Since no data on the constituents of citrus woods were available, this investigation was undertaken.

Materials and methods

Bole-wood samples (two from each tree) were collected from single trees of healthy Valencia orange, Washington Navel orange, and Marsh grape-

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fruit on sweet- and on sour-orange rootstock, and from Eureka lemon on sweet-orange and on Rough-lemon rootstock. Samples were also obtained from four psorosis(virus)-infected Valencia orange trees on sour-orange rootstock. Two of these trees showed leaf symptoms of the disease only, but through budding experiments they were known to contain the virus; the other two trees showed severe bark symptoms.

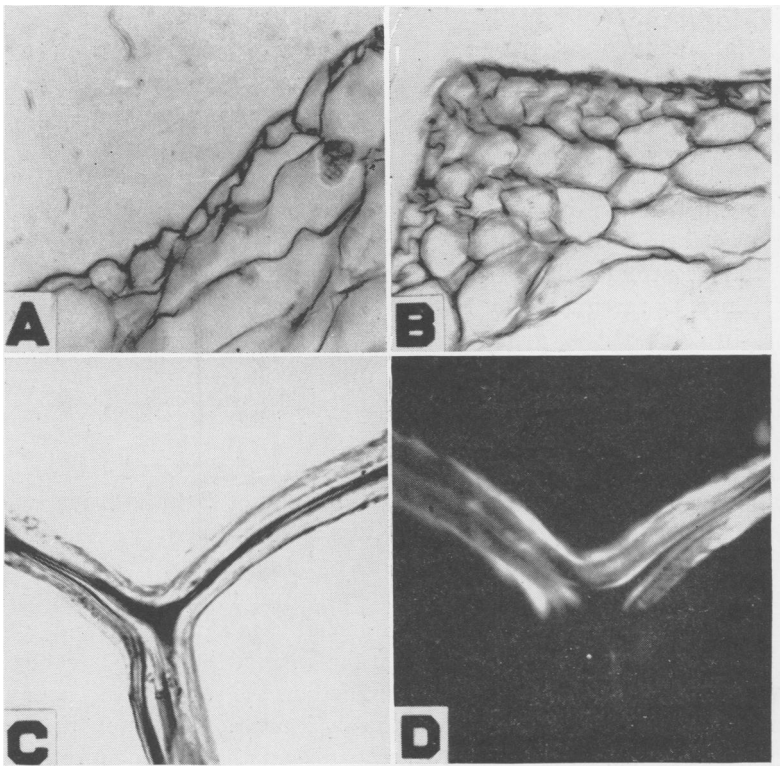

FIG. 1. Cell walls of normal and granulated Valencia orange juice vesicles. A. Thin cell walls along the edge of a normal juice vesicle (×336). B. Thin cell walls at the corner of a normal juice vesicle (×336). C. Thick cell walls found in the center of a granulated juice vesicle (×336). D. Same cell walls from the center of a granulated juice vesicle, photographed under polarized light. The double refractive cellulose is bright, while the lignified portions of the wall are nonrefractive and dark.

The samples, consisting of pieces $\frac{3}{4}$ inch × $\frac{3}{4}$ inch × 5 inches in size, were cut vertically from the sapwood of the scion portion of the tree trunk about $1\frac{1}{2}$ feet above the ground. Twigs were selected from two healthy Valencia orange trees on sour-orange rootstock, where the trunk wood was not sampled.

All trees were located in the experimental plots at the Citrus Experiment Station and were approximately twelve years old, except the psorosis(virus)-infected Valencias, which may have been somewhat younger.

The bark, which was shown by tests with $FeCl_3$ on water extracts to contain tannins, was stripped off all samples except the twigs before the wood was splintered and dried. The air-dried wood was then ground in a Wiley mill until fine enough to pass through an 80-mesh sieve and was dried at 105° C. for 48 hours in thin layers in shallow aluminum drying dishes. Forty-eight hours was determined to be the shortest drying time required to obtain practically constant weight. Longer drying periods gave satisfactory results up to that of 120 hours, when there were additional slight losses of weight.

In drying woods other than citrus, PARR and DAVIDSON (**10**) found that volatile constituents other than water accounted for a portion of the loss in dry weight. In the light of their findings, it is probable that volatile oils are also lost in drying citrus woods and must be included in the moisture lost. Since, as recognized by PARR and DAVIDSON, dry weight is important in certain calculations, great care must be exercised in obtaining a satisfactory working value. While every precaution was used, much is yet to be desired in this determination, especially with reference to the ash constituents.

The proximate inorganic constituents were determined on the ash of thoroughly oven-dried ground wood. Samples of 2 to 3 grams of wood were weighed into tared porcelain crucibles and ignited over a Bunsen flame. When the ash began to whiten (after 20 to 30 minutes), the crucibles were transferred to a muffle furnace heated to a dull red, and combustion was continued 4½ hours. They were then cooled in a desiccator and weighed. The percentage of ash was calculated on the basis of the weight of the sample of oven-dried wood. Care was used in keeping the samples the same length of time in the drying ovens and in the desiccator. Although the samples were in tightly closed aluminum drying dishes, the woods were so highly hygroscopic that they gained weight in the desiccator and during the weighing.

Spectrograms were made by volatilizing the ash of the various woods in a carbon arc and passing the light produced through a diffraction-grating spectrograph equipped with a rotating shutter. In order to make the intensities of the lines of the elements comparable between pairs of samples and to indicate the relative exposures during the arcing of the sample, minute amounts of the salts of thallium, cadmium, cobalt, and beryllium were introduced into the ash samples as controls. Preliminary analyses using these added salts indicated that they did not appreciably alter the concentration of elements present in the samples. Moving-plate studies were made

of some of the samples to obtain an estimate of the quantities of elements present in the samples, the cycles at which they were volatilized, and the relation of these cycles to the cycles at which the elements added for exposure control were volatilized. The same reference standard was used for healthy Valencia orange scions on sweet- and on sour-orange rootstocks. A different reference standard was prepared for Washington Navel orange scions on sweet- and on sour-orange rootstocks. Marsh grapefruit on sweet-orange was referred to the same standard as Marsh grapefruit on sour-orange rootstock. The Eureka lemon scion on sweet-orange rootstock was referred to the same standard as Eureka lemon on Rough-lemon rootstock. The healthy twigs, psorosis-infected trunk woods, and the healthy Valencia orange scion on sour-orange rootstock were all referred to the same standard and are therefore comparable, although the accuracy is lower than for the individual comparisons since the photographic film gamma rating varies slightly from film to film.

The distance between the points at which a spectral line image fades out (L) was measured with a projector comparator employing a ground glass screen. Where L_1 and L_2 are two such values, the intensities of the lines (I_1, I_2) are related to them as indicated in the equation $L_1/L_2 = I_2/I_1$, as pointed out by VANSELOW and LAURANCE (**17**).

Tannin tests were made with $FeCl_3$ on water extracts and on 95 per cent. ethyl-alcohol extracts of the ground wood. The results were consistently negative in all the woods used except that of the twigs; these showed a positive test. Pretreatment with 95 per cent. ethyl alcohol, as recommended for catechol tannins by RITTER and BARBOUR (**13**) was found unnecessary for the trunk-wood samples.

The method of proximate analysis of the various woods was adapted from that used by GOSS and PHILLIPS (5) in the "preparation" of the wood for the lignin assay. Our adaptation is described in detail as follows: 1.5-gram samples of the ground wood were weighed into cellulose thimbles, 65×22 mm., which were placed in the siphon cups of A. S. T. M. apparatus commonly used in the analysis of rubber products. This apparatus consisted of a block-tin condenser and a 400-ml. Pyrex flask. It was very efficient, losing less solution than the SOXHLET extractors in equal periods of operation. The extraction was made for 6 hours with 150 ml. of 32 parts by weight of 95 per cent. ethyl alcohol and 68 parts by weight of benzene. After extraction, the thimbles were dried in air overnight, and samples were removed to weighing bottles by means of a glass stirring rod; care was taken not to scrape the cellulose loose from the thimble. The weighing bottles with included samples were dried in an oven at 105° C. to remove the last traces of moisture and solvent, cooled in a desiccator for 20 minutes, and weighed. The loss in weight of the samples represented the first group of substances removed and included fats, waxes, resins, and oils.

The second group of substances removed consisted of water-soluble pectins, sugars, and ash. The samples were transferred to 500-ml. Erlenmeyer flasks and boiled with 225 ml. of distilled water under a HOPKINS reflux condenser for 3 hours. The mixture was filtered hot; in order to insure against lowering of the temperature, a jacket of heavy, coiled copper wire was placed around the filtering crucible and heated with a Bunsen flame. The sample was washed thoroughly with hot water.

In the preliminary analyses, sintered-glass crucibles were used. These were replaced with Alundum crucibles (R. A. 98 porosity), however, after it was ascertained that the latter could be used satisfactorily. The Alundum crucible was ignited at a high temperature in a muffle furnace for several hours to oxidize the organic matter. It was then cooled and washed with distilled water, heated for 3 hours in an oven at 105° C., cooled for 20 minutes in a desiccator, and was weighed within 2½ minutes. The same sample was always filtered in the same crucible. When a new sample was used, the crucible was prepared in the manner described; heated for 3 hours at 105° C., cooled for 20 minutes, and reweighed before the new sample was filtered.

The acid-hydrolyzable polysaccharides, consisting of protopectins and hemicelluloses, made up the third group of substances removed from the wood samples. These substances were hydrolyzed with 225 ml. of 1 per cent. HCl by boiling the samples in 500-ml. Erlenmeyer flasks under reflux condensers for a 3-hour period. While still hot, the samples were filtered through the same crucibles used in the previous filtration. The samples were washed with hot distilled water until free of acid, as determined by adding $AgNO_3$ to the wash water. The crucible and contents were dried at 105° C. for 3 hours, cooled in a desiccator for 20 minutes, and weighed.

The fourth group of constituents, which consisted largely of cellulose, was removed from the wood samples by hydrolysis with fuming HCl (sp. gr. 1.210) at 8° C. The method followed was that of GOSS and PHILLIPS (**5**) for determining lignin, except that the alcohol-benzene extraction time was limited to 6 hours, as recommended in PHILLIPS'S earlier work (**11**).

After the cellulose extraction, the lignin was refluxed in the diluted acid, filtered through the Alundum crucible corresponding to the sample number, and washed until the test water gave a negative test with $AgNO_3$. The lignin was dried at 105° C. for 3 hours, cooled in a desiccator for 20 minutes, and weighed. It was then transferred to a porcelain crucible and ashed at 900° C.

Microanalyses were made on some samples by the KJELDAHL method with the GUNNING-ARNOLD modification. As the amount of nitrogen was small and nearly constant, corrections of the percentage of lignin for nitrogen were not made.

Analyses of the lignin content of citrus woods were also made according to the method of RITTER and BARBOUR (**13**). In this method 72 per cent. H_2SO_4 was employed to remove the cellulose. Hydrolysis was begun with the acid precooled to 20° C. $BaCl_2$ was used in the wash water to test for the complete removal of the SO_4^{--}. Alundum crucibles were employed which necessitated ash corrections.

Results

The results of 25 analyses for the ash of citrus scions, 12 spectrographic analyses for 14 elements in the ash, 28 analyses each for alcohol-benzene extractable substances, water-extractable substances, and 1 per cent. HCl-hydrolyzable substances, 30 analyses for fuming-HCl-hydrolyzable substances, and 54 analyses for lignin are reported. These analyses are based on samples of the trunk wood of scions of eight different healthy trees on three different types of rootstock, four psorosis(virus)-infected scions on one type of rootstock, and twigs from two healthy scions on one type of rootstock. The results reported as percentage of the dry weight of the samples of ground wood are the average of closely agreeing duplicate analyses. The preparation losses are not corrected for ash but the lignin values have been corrected.

Reference to table I shows that in the trunkwoods the healthy wood of Marsh grapefruit scions contained the highest percentage of ash, that the Valencia and Washington Navel orange woods contained an intermediate percentage, while the Eureka lemon wood contained the lowest percentage of ash. The psorosis-infected woods averaged a slightly higher percentage of ash than did healthy Valencia orange trunk wood; those with bark symptoms containing higher percentages of ash than those without. Twigwood (with bark) contained a much higher percentage of ash than did the trunk woods.

Valencia orange, Washington Navel orange, and Marsh grapefruit scions on sour-orange rootstock contained a lower percentage of ash than such scions on sweet-orange rootstock. The Eureka lemon scion on sweet-orange rootstock contained a higher percentage of ash than that on Rough-lemon rootstock. The smaller differences shown in table I are not significant.

Spectrographic analyses of the ash of the citrus woods are shown in table I. The values express ratios of light intensities emitted by the elements in the samples compared. The light intensities recorded are related to the concentration of the elements from which the light is emitted by a function of several variables. The exact relationship for our material and our conditions of excitation were not available and were not determined. Though the line relating concentration and light intensity is probably curvilinear, if points on the curve are selected close together, the relation between the points is approximately linear. In this work the concentra-

TABLE I

PROXIMATE INORGANIC CONSTITUENTS OF WOODS OF HEALTHY AND PSOROSIS-INFECTED CITRUS SCIONS ON VARIOUS ROOTSTOCKS. TOTAL ASH EXPRESSED AS PERCENTAGE OF DRY WEIGHT OF WOOD. APPROXIMATE RELATIVE CONCENTRATION OF ELEMENTS IN ASH OF PAIRS OF WOOD SAMPLES DETERMINED SPECTROGRAPHICALLY AND SHOWN AS THE RATIO OF LIGHT INTENSITIES EMITTED BY ELEMENTS OF ANY TWO WOODS COMPARED, THE VALUE OF THE DENOMINATOR IN EACH RATIO BEING 1

Wood designation	Scion	Rootstock	Percentage ash	Woods compared	Ratio of light intensities, indicating relative concentrations of													
					Na	K	Cu	Mg	Ca	Sr	Ba	B	Al	Si	Sn	Pb	Mn	Fe
Healthy trunk wood																		
			%															
a	Valencia orange	Sweet orange	1.53	a/b	0.92	1.00	1.00	1.00	1.15	1.15	0.96	0.77	0.96	1.15	0.16	0.24	1.00	1.15
b	Valencia orange	Sour orange	1.27	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
c	Washington Navel orange	Sweet orange	1.49	c/d	0.80	0.80	1.20	1.00	1.00	1.00	1.10	0.53	0.10	0.32	$\frac{0.00^*}{1.00}$	$\frac{0.00^*}{1.00}$	0.75	0.80
d	Washington Navel orange	Sour orange	1.09	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
e	Marsh grapefruit	Sweet orange	3.24	e/f	1.09	1.09	1.55	1.25	1.14	1.00	1.09	1.25	2.50	2.60	$\frac{0.00^*}{1.00}$	0.51	1.16	1.70
f	Marsh grapefruit	Sour orange	2.40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
g	Eureka lemon	Sweet orange	1.05	g/h	0.91	1.00	0.72	0.92	1.09	1.00	1.00	1.08	0.91	0.67	$\frac{0.00^*}{1.00}$	1.00	0.50	0.45
h	Eureka lemon	Rough lemon	0.65	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Psorosis-infected trunk wood (with bark symptoms)																		
i	Valencia orange Scion 1	Sour orange	1.64	i/b	0.92	1.12	1.00	1.15	1.20	1.00	1.05	0.70	0.63	0.80	0.51	0.55	0.91	1.00
	Scion 2	Sour orange	1.77	i/j	0.82	1.12	0.94	0.80	0.91	0.80	1.30	0.87	1.60	1.04	1.33	1.20	1.00	1.00
Psorosis-infected trunk wood (with no bark symptoms)																		
j	Valencia orange Scion 1	Sour orange	1.52	j/b	1.00	1.00	1.00	1.33	1.18	1.20	0.80	0.75	0.45	0.79	0.33	0.52	1.00	1.00
	Scion 2	Sour orange	1.13	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Healthy twig wood (with bark)																		
k	Valencia orange (2 scions)	Sour orange	5.97	k/b	0.63	0.88	1.00	1.20	1.37	1.10	1.13	0.74	0.86	0.93	0.33	0.57	0.86	0.60

* Ratios are shown, as the quotients would be meaningless. The ratios show that in scions on sweet-orange rootstocks tin was not detected, but in the scions on sour-orange rootstock tin was detected. Lead was not detected in Washington Navel orange scion on sweet-orange rootstock but was detected in this scion on sour-orange rootstock.

tion of elements was not greatly different. The ratio of the element concentration to the light intensity was, therefore, approximately linear. The light intensity values recorded are reciprocals of the lengths of the blank spaces between the emission lines. Thus a value lower than 1 indicates that the concentration of the element in the numerator is smaller than that in the denominator; and a value greater than 1, vice versa.

The results are probably accurate to ± 10 per cent. Thus, values differing by 20 per cent. or more will be considered significantly different. Comparisons are valid only for single elements between pairs of samples, as listed, except where several samples are referred to a common reference sample, as in the cases of comparisons *a* and *b*, *i* and *b*, *j* and *b*, *k* and *b* (table I), where it is possible to make comparisons between *a*, *i*, *j*, and *k*. In these cases the accuracy is less, because of variation in film gamma, and differences of 25 per cent. will be considered significantly different.

The ash samples from the healthy trunk wood of Washington Navel orange scion on sweet-orange rootstock (*c*) and Eureka lemon scion on Rough-lemon rootstock (*h*) were so small that the results could not be checked by duplicate determinations as in all of the other analyses. The shortage of material in these two samples made it necessary to use a compromise in exposure conditions in order to estimate the concentrations of all the elements in question in a single exposure.

The trunk-wood ash of healthy Valencia orange scion on sweet-orange rootstock contained a lower concentration of boron, tin, and lead than did the Valencia orange scion on sour-orange rootstock. Washington Navel scion on sweet-orange rootstock contained a lower concentration of sodium, potassium, boron, aluminum, silicon, manganese, tin, lead, and iron, and a higher concentration of copper than did Washington Navel orange scion on sour-orange rootstock. The internal standard applicable to aluminum and silicon shows low light intensities indicating that the light intensity values are low for this sample. Marsh grapefruit scion on sweet-orange rootstock contained a lower concentration of tin and lead and a higher concentration of copper, magnesium, boron, aluminum, silicon, and iron than Marsh grapefruit scion on sour-orange rootstock. Eureka lemon scion on sweet-orange rootstock contained a lower concentration of copper, silicon, manganese, and iron than Eureka lemon scion on Rough-lemon rootstock, as shown in table I.

The trunk-wood ash of psorosis-infected Valencia orange scion on sour-orange rootstock, having bark symptoms, contained lower concentrations of boron, aluminum, silicon, tin, and lead, and higher concentrations of calcium than the trunk-wood ash of healthy Valencia orange scion on sour-orange rootstock. Psorosis-infected Valencia orange scion having bark symptoms contained a lower concentration of magnesium and strontium, and

a higher concentration of barium, aluminum, tin, and lead than psorosis-infected Valencia orange scion not having bark symptoms. The psorosis-infected Valencia orange scion not having bark symptoms contained a lower concentration of barium, boron, aluminum, silicon, tin, and lead, and a higher concentration of magnesium and strontium than healthy Valencia orange scion on sour-orange roostock.

Twig wood (with bark) of healthy Valencia orange scion on sour-orange rootstock contained a lower concentration of sodium, boron, tin, lead, and

TABLE II

PROXIMATE ORGANIC CONSTITUENTS OF WOODS OF HEALTHY AND PSOROSIS-INFECTED CITRUS SCIONS ON VARIOUS ROOTSTOCKS, DETERMINED AS PREPARATION LOSSES FOR THE PHILLIPS HCl METHOD FOR LIGNIN AND EXPRESSED AS PERCENTAGE OF DRY WEIGHT. VALUES ARE MEANS OF DUPLICATE ANALYSES

Scion	Rootstock	Percentage loss through: Alcohol-benzene extraction	Water extraction	1 per cent. HCl hydrolysis	Fuming HCl hydrolysis
Healthy trunk wood					
		%	%	%	%
Valencia orange	Sweet-orange	5.64	12.07	32.28	35.74
Valencia orange	Sour-orange	8.43	12.14	32.97	33.51
Washington Navel orange	Sweet-orange	4.09	4.33	34.51	40.80
Washington Navel orange	Sour-orange	5.19	7.12	33.17	37.49
Marsh grapefruit	Sweet-orange	4.15	4.96	35.75	38.84
Marsh grapefruit	Sour-orange	7.04	8.67	36.91	31.60
Eureka lemon	Sweet-orange	4.73	5.94	34.09	38.77
Eureka lemon	Rough-lemon	3.57	4.70	30.99	43.04
Psorosis-infected trunk wood (with bark symptoms)					
Valencia orange					
Scion 1	Sour-orange	7.48	12.80	35.17	31.85
Scion 2	Sour-orange	5.46	10.63	35.99	32.30
Average		6.47	11.71	35.58	32.07
Psorosis-infected trunk wood (with no bark symptoms)					
Valencia orange					
Scion 1	Sour-orange	4.87	13.83	36.86	32.62
Scion 2	Sour-orange	5.18	10.97	34.16	34.51
Average		5.02	12.40	35.51	33.56
Healthy twig wood (with bark)					
Valencia orange					
Scion 1	Sour-orange	12.96	13.78	32.93	28.61
Scion 1	Sour-orange	.....	.....	.....	29.92
Scion 2	Sour-orange	11.50	9.54	31.81	34.02
Average		12.23	11.66	32.37	30.85

iron, and a higher concentration of magnesium and calcium than healthy Valencia orange scion trunk wood on sour-orange rootstock.

Percentage loss in the alcohol-benzene extraction represents the aggregate amount of fats, waxes, resins, and oils in the various citrus woods. Reference to table II shows that in this extraction the percentage losses from the sour-orange rootstocks (8.43, 5.19, and 7.04) were higher than those from the sweet-orange rootstocks (5.64, 4.09, and 4.15) and that the sour-orange rootstocks therefore produced higher percentages of these substances than the sweet-orange rootstocks. Washington Navel orange on sour-orange rootstock yielded 1.10 per cent. more alcohol-benzene extractable constituents than Washington Navel orange on sweet-orange rootstock. Valencia orange on sour-orange rootstock yielded 2.79 per cent. more alcohol-benzene extractable constituents than Valencia orange on sweet-orange rootstock, and Marsh grapefruit on sour-orange rootstock yielded 2.89 per cent. more alcohol-benzene extractable constituents than Marsh grapefruit on sweet-orange rootstock.

Percentage loss in the hot water extraction (table II) represents the aggregate amount of water-soluble pectins, sugars, and ash in the various woods. In this extraction, the percentage losses of the scions on sour-orange rootstocks (12.14, 7.12, and 8.67) ranged from slightly higher to nearly double those of the sweet-orange rootstocks (12.07, 4.33, and 4.96). The Valencia orange scion was affected least by the rootstock, while the Marsh grapefruit was affected most.

Acid-hydrolyzable polysaccharides, as protopectins and hemicelluloses, are represented in the percentage loss on 1 per cent. HCl hydrolysis (table II). No regular trend was effected by the type of rootstock. Valencia orange and Marsh grapefruit showed slightly greater percentage losses of acid-hydrolyzable substances on sour-orange rootstock (32.97, and 36.91) than on sweet-orange (32.28, and 35.75); the Washington Navel orange showed a lower percentage on sour (33.17) than on sweet (34.51). In comparison, the small value representing the percentage loss (principally hemicelluloses) in the Eureka lemon on Rough lemon (30.99) is notable.

Cellulose is the main constituent represented by the loss in hydrolysis with fuming HCl. In this extraction, Valencia orange, Washington Navel orange, and Marsh grapefruit scions on sour-orange rootstock show smaller percentage losses of this cell-wall material (33.51, 37.49, and 31.60) than such scions on sweet-orange rootstock [(35.74, 40.80, and 38.84) (table II)]. It is interesting to note not only the very high percentage of cellulose found in Eureka lemon on Rough-lemon rootstock (43.04), as compared with that found in Eureka lemon on sweet-orange rootstock (38.77), but also this high value as compared with most of the other values obtained (table II). The high percentage of cellulose may be of especial significance. DORE (**4**) has

discussed critically the proximate analysis of woods and should be consulted as to its limitations.

The percentage of lignin determined by the PHILLIPS HCl method (table III) was higher for Marsh grapefruit on sweet-orange rootstock (16.24) than on sour (15.65). A larger percentage of lignin was found in

TABLE III

LIGNIN CONTENT OF WOODS OF HEALTHY AND OF PSOROSIS-INFECTED CITRUS SCIONS ON VARIOUS ROOTSTOCKS, DETERMINED BY THE PHILLIPS HCl AND BY THE RITTER H_2SO_4 METHODS AND EXPRESSED AS PERCENTAGE OF DRY WEIGHT, CORRECTED FOR ASH. VALUES ARE MEANS OF DUPLICATE ANALYSES

SCION	ROOTSTOCK	LIGNIN DETERMINATION	
		HCl METHOD	H_2SO_4 METHOD
HEALTHY TRUNK WOOD			
		%	%
Valencia orange	Sweet-orange	12.84	12.99
Valencia orange	Sour-orange	12.65	14.02
Average		12.74	13.50
Washington Navel orange	Sweet-orange	16.13	21.36
Washington Navel orange	Sour-orange	16.89	20.69
Average		16.51	21.02
Marsh grapefruit	Sweet-orange	16.24	21.27
Marsh grapefruit	Sour-orange	15.65	21.37
Average		15.94	21.32
Eureka lemon	Sweet-orange	16.18	21.82
Eureka lemon	Rough-lemon	17.35	20.96
Average		16.76	21.39
PSOROSIS-INFECTED TRUNK WOOD (WITH BARK SYMPTOMS)			
Valencia orange			
Scion 1	Sour-orange	11.60	14.67
Scion 2	Sour-orange	12.94	14.90
Average		12.27	14.78
PSOROSIS-INFECTED TRUNK WOOD (WITH NO BARK SYMPTOMS)			
Valencia orange			
Scion 1	Sour-orange	11.43	16.86
Scion 2	Sour-orange	13.46	17.70
Average		12.44	17.28
HEALTHY TWIG WOOD (WITH BARK)			
Valencia orange			
Scion 1	Sour-orange	11.55	.....
Scion 1	Sour-orange	11.51	.....
Scion 2	Sour-orange	12.80	.....
Average		11.95	.....

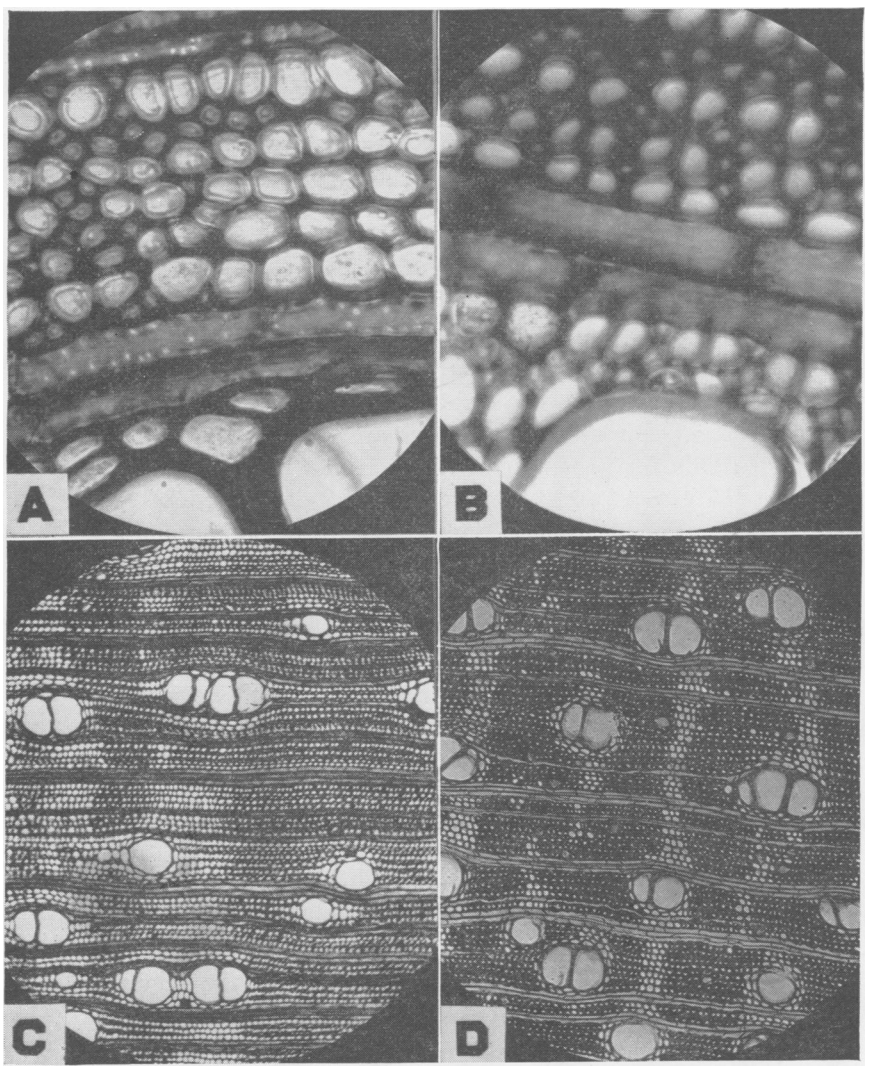

FIG. 2. A. Transverse section of Valencia orange wood showing the thick cell walls (fibers, 5.1 μ in width; tracheids, 3.2 μ; vessels, 4.8 μ). The walls are composed of a large portion of clear unstained material (higher carbohydrate) and a small portion of safranin stained material (lignin). (×518.) B. Transverse section of Washington Navel orange wood showing comparatively thin cell walls (fibers, 4.5 μ in width; tracheids, 2.2 μ; vessels, 5.8 μ). The walls show almost no material unstained with safranin. (×518). Transverse sections of Valencia orange wood (C) and of Washington Navel orange wood (D.) (Both ×57.) Because of the smaller proportion of fibers, the wood of the former is more porous than that of the latter.

the Washington Navel orange on sour-orange rootstock (16.89) than on sweet (16.13). The high percentage of lignin in the Eureka lemon on Rough-lemon rootstock (17.35), as compared with the percentage of lignin in the Eureka lemon on sweet-orange rootstock (16.18) or as compared with the other lignin values, is notable. The percentage of lignin was lower in Valencia orange wood than in the other citrus woods analyzed. Microscopic examination of sections of Valencia orange and Washington Navel orange woods shows that the cell walls of the former are thicker than those of the latter, but the cell walls of the Valencia orange do not show such wide bands of safranin stain (fig. 2) as those of the Washington Navel orange. This suggests that the Valencia wood contains less lignin.

Assay of lignin by the RITTER H_2SO_4 method (table III) shows a relatively larger value in every instance in which the HCl method shows a smaller value, when rootstocks are compared. Comparison of mean percentages of lignin for woods of the different varieties and species, however, shows that the general trend is practically the same, whichever method is used. The one exception is in the case of the Washington Navel orange wood, which shows a higher percentage of lignin (16.51) than Marsh grapefruit wood (15.94) when the HCl method is used, but a lower percentage of lignin (21.02) than Marsh grapefruit wood (21.32) when the H_2SO_4 method is used.

Valencia orange wood (scion) from psorosis-infected trees grown on sour-orange rootstock, when analyzed, gave more variable results than the healthy woods. This variation is somewhat obscured in the results reported in table II, since these are averages of duplicate analyses. Alcohol-benzene extractable materials composed a higher percentage of the dry weight of the wood of those scions having bark symptoms of the disease than of those having no bark symptoms, but this difference is not significant. No clear trend of the proximate constituents was evident in the water extraction or in the 1 per cent. HCl hydrolysis. The percentage of materials hydrolyzed with fuming HCl was slightly lower, however, in the scions with bark symptoms, but this difference is not significant.

Psorosis-infected woods of scions with or without bark symptoms contained lower percentages of alcohol-benzene extractable substances and higher percentages of 1 per cent. HCl-hydrolyzable substances than healthy Valencia scions. Scions with bark symptoms contained less fuming-HCl-hydrolyzable material than healthy scions. No clear trend was noted for the water-extractable materials.

In all cases, duplicate samples of wood from scions having psorosis symptoms in the bark varied from 2 to 3 per cent. in lignin content, as determined by the PHILLIPS HCl and by the RITTER H_2SO_4 methods. One set of samples from infected scions showing no bark symptoms gave similar

TABLE IV

F AND t VALUES FOR COMPARISONS TESTED FOR SIGNIFICANCE OF DIFFERENCE BY ANALYSIS OF VARIANCE AND "t" TEST

COMPARISONS	F	t
PERCENTAGE OF ASH (HEALTHY WOOD)		
	%	%
Marsh grapefruit, both rootstocks *vs.* Valencia orange, both rootstocks	25.605‡	.....
Valencia orange, both rootstocks *vs.* Eureka lemon, both rootstocks	13.023*	.....
Washington Navel orange, sweet-orange rootstock *vs.* Washington Navel orange sour-orange rootstock	.....	.....
Marsh grapefruit, sweet-orange rootstock *vs.* Marsh grapefruit, sour-orange rootstock	8.301†	27.184‡
Valencia orange, sweet-orange rootstock *vs.* Valencia orange, sour-orange rootstock	75.111*	.....
Eureka lemon, sweet-orange rootstock *vs.* Eureka lemon, Rough-lemon rootstock	5.861†	10.256‡
PERCENTAGE OF ASH (DISEASED WOOD)		
Psorosis-infected Valencia orange (bark symptoms), sour-orange rootstock *vs.* healthy Valencia orange, sour-orange rootstock	12.620*	.....
Psorosis-infected Valencia orange (no bark symptoms), sour-orange rootstock *vs.* healthy Valencia orange, sour-orange rootstock	0.000†	1.795†
Psorosis-infected Valencia orange (bark symptoms), sour-orange rootstock *vs.* psorosis-infected Valencia orange, sour-orange rootstock (no bark symptoms)	9.471*	.....
Valencia orange, sour-orange rootstock trunk wood *vs.* Valencia orange, sour-orange rootstock, twig wood	57.179‡	.....
PERCENTAGE OF ORGANIC CONSTITUENTS (HEALTHY WOOD)		
Alcohol-benzene soluble constituents Valencia orange, Washington Navel orange, Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	10.523‡	.....
Water-soluble constituents Valencia orange, Washington Navel orange, Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	1.312†	15.089‡
Dilute HCl hydrolyzable constituents Eureka lemon, sweet-orange rootstock *vs.* Eureka lemon, Rough-lemon rootstock	61.170*	.....
Fuming HCl hydrolyzable constituents Valencia orange, Washington Navel orange, Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	8.444*	.....
Fuming HCl hydrolyzable constituents Eureka lemon, sweet-orange rootstock *vs.* Rough-lemon rootstock	14.532†	21.616‡
Fuming HCl hydrolyzable constituents Eureka lemon, Rough-lemon rootstock *vs.* Valencia orange, Washington Navel orange, Marsh grapefruit, Eureka lemon on sweet-orange rootstock	7.541*	.....
Lignin Valencia orange, sweet-orange rootstock *vs.* sour-orange rootstock	0.696†	1.348†
Lignin Washington Navel orange, sweet-orange rootstock *vs.* sour-orange rootstock	23.385*	.....
Lignin Eureka lemon, sweet-orange rootstock *vs.* Rough-lemon rootstock	8.052†	8.298‡
Lignin Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	8.681†	4.184‡
Lignin Valencia orange, both rootstocks *vs.* Washington Navel orange, Marsh grapefruit, Eureka lemon, both rootstocks (lemon on sweet only)	130.205‡	.....
Lignin all scions on all rootstocks, HCl method *vs.* all scions on all rootstocks, HCl method	86.714‡	.....
Lignin all scions on all rootstocks, H_2SO_4 method *vs.* all scions on all rootstocks H_2SO_4 method	28.655‡	.....

TABLE IV—(*Continued*)

Comparisons	F	t
Percentage of organic constituents (diseased wood)		
	%	%
Alcohol-benzene soluble constituents psorosis-infected Valencia orange, bark symptoms *vs.* no bark symptoms	0.010†	0.794†
Alcohol-benzene soluble constituents all psorosis-infected Valencia orange, sour-orange rootstock *vs.* Valencia orange healthy, sour-orange rootstock	17.263‡	.....
Dilute HCl hydrolyzable constituents all psorosis-infected Valencia orange, sour-orange rootstock *vs.* Valencia orange healthy, sour-orange rootstock	13.120‡	.....
Fuming HCl hydrolyzable constituents psorosis-infected Valencia orange, bark symptoms *vs.* no bark symptoms	2.940†	1.869†
Alcohol-benzene soluble constituents Valencia orange (trunkwood), sour-orange rootstock *vs.* Valencia orange (twigwood), sour-orange rootstock	27.289‡	.....
Lignin Valencia orange (trunkwood) sour-orange rootstock *vs.* Valencia orange (twigwood), sour-orange rootstock	7.245*	.....
Lignin, all scions, all rootstocks, HCl method *vs.* all scions, all rootstocks, H_2SO_4 method	15.597‡	.....

‡ Highly significant; * Significant; † Not significant.

variability with the two methods of assay, while a second set of samples gave consistent results with both methods. The averages of the percentages of lignin in each scion in the psorosis (virus)-infected Valencia wood (table III) agree fairly well with those found for healthy wood (table III) except in the case of wood samples from scions showing no bark symptoms, assayed by the RITTER H_2SO_4 method. The average percentage of lignin in these samples is 3.2 per cent. higher than that of the healthy Valencia wood assayed by the same method. In all analyses (table III), the RITTER H_2SO_4 method yielded higher percentages of lignin than the PHILLIPS HCl method.

Analysis showed that twig wood (including bark) from healthy Valencia orange scions on sour-orange rootstock (table II) contained a higher percentage of alcohol-benzene extractable substances than trunk wood. There is no clear trend in the substances lost in water extraction, in hydrolysis with 1 per cent. HCl, or in hydrolysis with fuming HCl.

The lignin analyses of twigs of Valencia orange scions on sour-orange rootstock are also shown in table III. The mean percentage of lignin in the twigs, as determined by the PHILLIPS HCl method, is 11.95. This value is 0.70 per cent. lower than the mean of similar assays by the PHILLIPS HCl method of the trunk-wood samples taken just above the bud union (table III).

Discussion and conclusions

OLIVERI and GUERRIERI (**9**), working in Italy, found that green, tender orange wood contained 40.99 grams of ash per 1,000 parts (4.10 per cent.);

old, hard orange wood contained 43.89 grams per 1,000 parts (4.39 per cent.). Green, tender lemon wood was found to contain 41.94 grams per 1,000 parts (4.19 per cent.), while old, hard lemon wood contained 45.68 grams per 1,000 parts (4.57 per cent.). The authors do not state the variety of orange or lemon used, the age of the trees, the exact portions of the tree from which the old, hard wood was obtained, nor whether bark was included. The fact that the percentage of ash for the old, hard orange wood is of approximately the same magnitude as our values for twigs with bark suggests that old twigs were used for analysis.

BLAIR (**2**), working in Florida, reported that twigs of very young (late) Valencia orange scions on sour-orange rootstock (trees in the field, 1909 to 1910) assayed from 3.83 to 4.57 per cent. ash. These values are slightly lower than the values we have found for Valencia orange twigs on older trees in California. Since neither BLAIR nor OLIVERI and GUERRIERI stated the length of time the wood was dried or the length of time the samples remained in the desiccator, it is impossible to say whether the differences noted in our results are actual differences in the ash content or merely differences due to the higher water content of their samples. Citrus woods are extremely hygroscopic, a 4- or 5-gram sample absorbing as much as 200 mg. of moisture from a $CaCl_2$-charged desiccator in 24 hours if the wood is thoroughly dried.

The differences in habit of growth exhibited by citrus scions and fruit of various species and varieties on different rootstocks, generally observed in the field, are a reflection of the different inorganic and organic constitution of the scions. Various portions of the same scion have different percentage compositions, as shown by comparing twig and trunk samples. This suggests that a virus disease also affects the percentage composition of the scions.

Valencia orange, Washington Navel orange, and Marsh grapefruit scions on sweet-orange rootstocks accumulated a higher percentage of ash in the trunk wood than did these scions on sour-orange rootstocks. The Eureka lemon scion on sweet-orange rootstock accumulated significantly more ash in the wood than did Eureka lemon on Rough-lemon rootstock.

Psorosis-infected scions with bark symptoms contained a slightly higher percentage of ash than did trunk wood of healthy Valencia orange scions. Infected scions with no bark symptoms gave inconclusive results, one scion assaying a higher percentage and one a lower percentage of ash than the healthy scions. Twig wood (with bark) had a higher percentage of ash than trunk wood of Valencia orange scions.

While the scion-rootstock combinations showed, in a general way, approximately the same relative concentration of elements in the ash, in some cases it seemed that there may have been selective absorption of elements, as in-

dicated by higher accumulations of certain metals. The more marked instances are the following: the lower accumulation of tin and lead by healthy Valencia orange scion on sweet-orange rootstock than by the same scion on sour-orange rootstock; the lower accumulation of aluminum, silicon, tin, and lead by Washington Navel orange scion on sweet-orange rootstock than by the same scion on sour-orange rootstock; the higher accumulation of aluminum and silicon and lower accumulation of tin and lead by Marsh grapefruit scion on sweet-orange rootstock than by the same scion on sour-orange rootstock; and the lower accumulation of manganese and iron by Eureka lemon scion on sweet-orange rootstock than by the same scion on Rough-lemon rootstock. In each of these cases one scion-rootstock combination contained elements in a concentration two or more times that of the one with which it has been compared. Though every precaution was taken to eliminate contamination of the samples with dust, it is possible that these differences may be due to such contamination or to traces of metals accumulated in grinding.

Psorosis-infected trunk wood of Valencia orange scion on sour-orange rootstock, where bark symptoms were visible, showed relatively low accumulations of boron, aluminum, silicon, tin, and lead, as compared with the ash of a healthy scion of the same kind on a similar rootstock. This comparison is closely approximated in the psorosis-infected Valencia orange scion having no visible bark symptoms, indicating that the accumulation of inorganic ions is probably related to the general vigor of the tree.

While healthy twig wood (with bark) showed concentrations of several elements in the ash to be different from those in the ash of healthy trunk wood of Valencia orange scion on sour-orange rootstock, tin was the element which was present in the lowest relative concentration. The higher concentration of magnesium in the twig sample may be explained by the chlorophyll content of the bark of the twig.

The various citrus scions on sour-orange rootstock gave higher percentages of alcohol-benzene extractable materials, and higher percentages of water-extractable substances than the citrus scions on sweet-orange rootstock.

Extractions with dilute (1 per cent.) HCl indicated that substances hydrolyzed by HCl were not closely related to the type of rootstocks, since no clear trend was noted for the various citrus scions. The scions on sweet-orange rootstock, however, gave higher percentages of substances removable by the fuming HCl (probably largely cellulose) than those on sour-orange rootstock.

All percentages of lignin reported are corrected for ash since a spectrographic analysis (fig. 3) showed that a large percentage of ash in the lignin was aluminum contributed by the Alundum crucibles. The strikingly heavy

lines in the spectrogram (fig. 3, A) are aluminum lines indicating the presence of an amount of aluminum far in excess of the aluminum normally present in citrus woods.

The mean error in the determination of lignin, in healthy woods by the PHILLIPS HCl method was 0.31 per cent.; that in the determination by the RITTER H_2SO_4 method, 0.80 per cent. An analysis of variance showed that the individual percentages of lignin obtained by either the PHILLIPS or the RITTER method, and recorded for each species or variety, are significantly different. How much individual tree variability may affect these results cannot be accurately estimated at the present time, although comparison of analyses of two of the psorosis-infected trees (table III) suggests that the variability may be as much as 2 per cent.

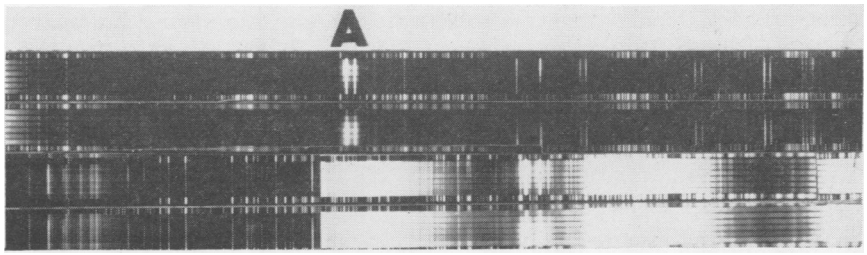

FIG. 3. Arc spectrograms of the ashes of lignin obtained by analyses with the HCl method (upper row of each pair) and with the H_2SO_4 method (lower row of each pair), showing the intense aluminum lines at A.

CARYL (3) has pointed out that 48 strains and variations of the Washington Navel orange, 47 of the Valencia orange, 9 of the Marsh grapefruit, and 12 of the Eureka lemon are now grown in California. There are also wide variations in soil, water, and climatic factors, as well as in cultural practices. Data on which to base an opinion of the effect of these factors on the percentages of constituents of citrus woods are not available; we feel, however, that they very likely do have an effect. When, in addition, the widely different areas in which citrus is grown in California are considered, the problem of adequate sampling as a basis of generalization is at once evident.

In the Riverside area, the Washington Navel orange scions on sweet- and on sour-orange rootstocks have a tendency toward greater cellulose formation and lignification than do Valencia orange scions on similar rootstocks. Eureka lemon scions on sweet-orange and Rough-lemon rootstocks and Marsh grapefruit scions on sweet-orange rootstocks also have a tendency to produce greater percentages of cellulose and lignin than the Valencia orange scions on sweet and sour rootstocks. Marsh grapefruit scions on sour-orange rootstock produce less cellulose than Valencia orange on sweet or sour root-

stocks; on either rootstock, however, they produce a higher percentage of lignin than do Valencia orange scions on either rootstock. Except for the lower production of cellulose by the Marsh grapefruit scions on sour-orange rootstock, these comparisons suggest that Valencia orange is a relatively low producer of cellulose and lignin.

The Washington Navel orange in the Riverside area sets its fruit in the spring, and the mature fruit is generally picked before a new crop is set. This variety matures its fruit rapidly, and if the fruit is allowed to remain on the tree for a few months after the new crop is set, it is occasionally granulated; that is, the sap gelates and the cell walls thicken and lignify. In California, granulation in navel oranges is not a matter for serious consideration, but in some countries the fruit of this variety granulates so badly that it cannot be grown for commercial purposes, especially on certain rootstocks. The Valencia variety also sets its fruit in the spring but matures the fruit so slowly that a second crop is set and fairly well developed before the first is matured. The Washington Navel fruit is matured in about 10 months, and the Valencia fruit in about 13 months, although there are wide fluctuations in both cases. If the Valencia crop is not picked until late (latter part of September) it is often more or less granulated. This suggests a positive correlation between rate of fruit maturation and granulation (excessive production of cellulose and lignin in the fruit) and the tendency toward cellulose and lignin production of the stem.

It is also known that Valencia orange scions on Rough-lemon rootstocks are among the high producers of granulated fruit (**1**). Unfortunately, samples of Valencia orange wood (scions) on Rough-lemon rootstock could not be obtained, but since Eureka lemon on Rough-lemon rootstock produced a high percentage of cellulose and of lignin, it may be surmised that this might prove true of Valencia orange on Rough lemon also. Thus there is a suggestion in this comparison that production of high percentages of granuated fruit by Valencia orange scions on Rough-lemon rootstock may be found to be the result of a tendency of scion and rootstock combination to produce relatively high percentages of lignin in the trunk wood.

Some citrus observers have maintained that the problem of granulation is one of overmature fruit. This correlates with the general botanical observation that lignification of parenchymatous tissue may accompany maturation and that sclerenchymatous tissues contain a higher percentage of lignin at maturity. Lignification has been observed by WILSON (**19**) to occur in mature phloem of several types of plants, and such a process may well occur in the thin-walled cells of "overmature" citrus fruits.

HAAS and KLOTZ (**6**) showed that granulated Valencia oranges contained 6.13 per cent. ash, as compared with 5.16 per cent. in normal fruits, when the results were expressed as percentage of dry matter. The ash content

of wood of trees producing large percentages of granulated fruit might therefore be expected to be high.

In this connection it is of interest to note that the percentage of ash of the Eureka lemon scion on Rough-lemon rootstock is significantly lower than that of any of the other woods assayed, including Marsh grapefruit. HAAS and KLOTZ (**6**) showed, however, that Eureka lemon fruit pulp contained 4.61 per cent. ash, whereas Marsh grapefruit pulp averaged 3.37 per cent. This suggests that trees which accumulate little ash in the trunk wood may accumulate more ash in the fruits than trees accumulating larger amounts of ash in the wood, and that such a condition may be correlated with the high percentage of granulated fruit produced by Valencia orange scions on Rough-lemon rootstock.

While some environmental factors are known to accelerate the maturation of plants, the factors which influence lignification are not well known, nor are the factors which accelerate granulation in citrus fruits.

The irregular results obtained in the analysis of psorosis-infected wood are, we believe, due to the ineffectual removal of gums shown by WEBBER and FAWCETT (**18**) to be formed in the vessels of the wood in this disease.

Psorosis evidently affects the normal metabolism of the Valencia scion. The diseased scions, both those with and those without bark symptoms, contained less alcohol-benzene extractable material and larger quantities of 1 per cent. HCl-hydrolyzable substances than healthy scions. The diseased wood having bark symptoms contained a lower percentage of fuming-HCl-hydrolyzable material. Water-extractable substances and lignin were not greatly affected by the presence of the virus.

Twig samples from the two scions of Valencia orange on sour-orange rootstock showed considerable variation. These samples are not strictly comparable to samples of the trunk wood since the bark was included in the former; but outstanding differences seem to be the higher percentage of ash and the higher percentage of alcohol-benzene extractable materials in the twig samples.

Comparisons of the results have been treated by analysis of variance in order to properly appraise the significance of such comparisons. In a number of cases the number of degrees of freedom was small; thus the analysis of variance indicated that the difference was not significant. The "t" test was applied in these cases in order to more critically judge the significance of the difference. In applying the "t" test, it was essential to calculate the standard errors of the various analyses. The standard errors in per cent. were: ash, 0.039; alcohol benzene soluble constituents, 0.214; water soluble constituents, 0.145; dilute HCl hydrolyzable constituents, 0.239; concentrated HCl hydrolyzable constituents, 0.198; lignin, 0.141. The various

comparisons made in this paper are listed in table IV with the "F" and "t" values and their significance rating.

Our results, obtained by the PHILLIPS HCl and the RITTER H_2SO_4 methods, were different and accord with the findings of other investigators. The difference is perhaps due to the action of H_2SO_4 on certain sugars such as xylose, fructose, arabinose (to a small extent), and polysaccharides containing pentose sugars which form an insoluble residue with 72 per cent. H_2SO_4 and increase the apparent lignin percentage (**8**). NORMAN (**7**) also showed that aldehydes, phenols, and nitrogenous substances of various types condense with 72 per cent. H_2SO_4 to give high apparent lignin values. PHILLIPS and GOSS (**12**) have made comparative studies on the HCl method and other methods of determining lignin. They have listed factors affecting the determination of lignin by the HCl method, as RITTER, SEBORG, and MITCHELL (**14**) and SHERRARD and HARRIS (**15**) have done for the H_2SO_4 method.

It is not in the province of this paper to reappraise the methods, but we would call attention to the fact that in our hands the error between duplicates was smaller with the HCl method; therefore, we have felt that it was more reliable. What the lignin values obtained by the two methods represent, however, is difficult to explain. For example, a sample of Aspen lignin prepared by the H_2SO_4 method in another laboratory was rerun by us by the PHILLIPS HCl and the RITTER H_2SO_4 methods. The preparation losses were approximately 25 per cent. and 20 per cent., and the yields of lignin approximately 65 per cent. and 80 per cent., respectively. There was a considerably larger loss of the apparent lignin value in the hydrolysis with fuming HCl than would be expected from the differences in assay by the two methods.

Summary

1. Lignin analyses were made on the sapwood of Washington Navel orange, Valencia orange, Eureka lemon, and Marsh grapefruit scions on sweet- and on sour-orange rootstocks, using the PHILLIPS HCl and the RITTER H_2SO_4 methods.

2. The proximate constituents represented by extraction losses both in alcohol-benzene and in hot water were higher for all the scions on sour-orange than for those on sweet-orange rootstocks and indicated higher percentages of oil and fatty substances, sugars, and pectins.

3. The percentage of constituents hydrolyzed by dilute (1 per cent.) HCl was lowest in wood from the Eureka lemon scion on Rough-lemon rootstock and highest in wood from the Marsh grapefruit scion on sour-orange rootstock. Valencia orange scions contained lower percentages of these con-

stituents than Washington Navel orange scions on either sweet- or sour-orange rootstocks.

4. The percentage of constituents hydrolyzed by fuming HCl was lowest in wood from the Marsh grapefruit scion on sour-orange rootstock and highest in wood from the Eureka lemon scion on Rough-lemon rootstock. Valencia orange scions contained lower percentages of these constituents than Washington Navel orange scions on either sweet- or sour-orange rootstocks.

5. The lignin content of various species and varieties of healthy citrus woods on various rootstocks assayed from 12.65 to 17.35 per cent. of the dry weight of the wood by the PHILLIPS HCl method and from 12.99 to 21.82 per cent. by the RITTER H_2SO_4 method.

6. Valencia orange scions contained the least lignin; Eureka lemon scions on Rough-lemon rootstock, the most. Psorosis-infected wood gave rather irregular results, although the average lignin content of this wood was about the same as that of healthy wood. Percentages of other constituents of the diseased wood differed from those of healthy wood.

7. Significantly higher values were obtained with the RITTER H_2SO_4 method than with the PHILLIPS HCl method.

8. The percentage of ash of the wood of healthy scions on sweet-orange rootstocks was higher than that of healthy scions on sour-orange rootstocks. Eureka lemon on Rough-lemon rootstock had the lowest percentage of ash. Psorosis-infected woods with bark symptoms had a slightly higher percentage of ash than healthy woods. Twig wood (with bark) contained a higher percentage of ash than trunk wood.

9. Spectrographic analyses of the ash of wood of healthy citrus scions of a given species showed that, in general, these scions contained about the same relative concentration of elements, whether on sweet-, sour-orange, or Rough-lemon rootstock. One or two specific elements, which varied from species to species, were found in low concentration in one or the other of the scion-rootstock combinations.

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