

CITRUS ROOTSTOCK IDENTIFICATION BY TISSUE

COLORING TESTS

by

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A Thesis

submitted to the faculty of the

Department of Horticulture

in partial fulfillment of

the requirements for the degree of

Master of Science

in the Graduate College

University of Arizona

1948

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August 12, 1948
Date

OFFICE OF THE DIRECTOR OF THE BUREAU OF RESEARCH

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ACKNOWLEDGMENT

Appreciation is extended to the Department of Horticulture of the University of Arizona for guidance during the period of study, for use of equipment and laboratory facilities, and for the encouragement that made this study possible. Especial credit is given to Dr. Leon M. Fultz and Dr. Leland Burkhart under whose supervision the study was conducted, particularly for their part in planning the experiment and reviewing the manuscript.

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INTRODUCTION

Commercial citrus propagation is practiced in the United States by budding the scion on a compatible rootstock. Most commonly used rootstocks at the present time are as follows:

1. Sour orange (Citrus aurantium) Linn.

Sour orange is a hardy deep-rooted stock, considered resistant to various fungus diseases especially to brownrot gummosis (2)¹. Until now, it was considered a compatible rootstock for oranges and grapefruit. In recent years, however, Washington navel and Valencia oranges on its top have shown in certain areas a virus disease called 'quick decline' which has caused a rapid decline or sudden death of the affected trees. So far it has affected only sweet orange on sour-orange root (2).

2. Rough lemon (Citrus limon) (Linn.) Burmann

Rough lemon rootstock produces large vigorous trees. It is suited to lighter soils, being used in soils where organic matter is low and topography rolling. The fruit, however, is low in both sugar and acid and often has a coarse texture and rather a thick rind (2). It also tends to favor development of granulation in the fruit, more especially in valencias (2). Both the tree and the fruit on this stock

1. Figures in parentheses refer to Literature Cited.

are more susceptible to cold, salt and drouth injury.

3. Sweet orange (Citrus sinensis) (Linn.) Osbeck

Sweet orange is adapted to a wide range of soils, but does best on soils that are light to medium loam in texture and is suitable for soil rich in organic matter and of level topography. In heavy soils this rootstock is susceptible to brownrot gummosis (2). It is, however, most widely used stock for all commercial varieties of citrus and is particularly suited to lemons.

4. Grapefruit (Citrus paradisi) Macf.

This has been used as a rootstock usually for lemons, and occasionally for oranges, but trees budded on it have proved less productive than on sweet or sour orange stocks (2). Consequently it is increasingly getting into disfavor among citrus growers.

The importance of identification of unknown rootstocks in old citrus groves cannot be overemphasized. In the first place this would offer us an excellent opportunity to study the interrelation between stock and scion, were it possible to evolve a definite 'knowhow' for the identification of these stocks. Secondly, it would help growers to fortify themselves against economic loss by obtaining trees on disease-resistant rootstocks for replacing the ones that may die of 'quick decline'. Thirdly, it would afford the citrus growers more reliable basis for starting new groves with plants raised on roots most compatible with particular soil and climatic conditions.

REVIEW OF LITERATURE

Hendrickson (4) published a method which is based on the fact that all citrus roots contain glucosides and that these glucosides contain phenols. He treated aqueous extracts of these roots with ferric chloride and observed different shades of color with sour orange, grapefruit and rough lemon. Sweet orange gave inconsistent results. This worker considers his method "reliable only for rough lemon".

Halma and Hans (4) report a series of tests which were conducted on powdered bark water-extracts of these stocks. Briefly stated, their methods were as follows:

a. Almen test:

Add 2 drops of KOH to 10 cc. of the water extract of the bark specimen under study, followed by 8 drops of saturated copper sulfate solution. Shake well. Then add 8 drops of Almen reagent². Boil the resulting heavy creamy yellow precipitate for about two minutes. This would result in the following color developments:

1. Sour orange brown
2. Rough lemon colorless
3. Grapefruit light pink
4. Sweet orange pink

2. Method of preparation of this as well as all other reagents are detailed on page 24.

"The sweet orange solutions show a narrow range of pink while the sour orange and grapefruit solutions fluctuate in some cases over a comparatively wide range within their color group."

b. Ammonium molybdate test:

Ten drops of this reagent added to 5 cc. of the liquid extract and boiled will give the following coloration.

1. Sour orange Varying shades of green
2. Rough lemon Deep blue color
3. Grapefruit Light blue or bluish gray
4. Sweet orange Varying shades of green color

c. Titanous chloride test:

Two drops of this reagent (20 % solution) added to 5cc. of the extract will yield the following coloration.

1. Sour orange Greenish color
2. Rough lemon Very light pink
3. Grapefruit Warm gray
4. Sweet orange Greenish color

d. Ferric chloride test:

Two drops of a saturated solution of this reagent added to 5 cc. of the bark extract will develop coloration as follows:

1. Sour orange Dark clear brown
2. Rough lemon Clear straw color
3. Grapefruit " " "
4. Sweet orange Brownish turbid

With regard to the last three test, the authors remark that "these methods will be found helpful, although they are not as reliable as the Almen reagent method".

L.C. Masters (5) reports on "A Review of Laboratory Tests for the Determination of Citrus Rootstocks", and has further developed the methods tried by Halma and Haas. He has endeavored to be more precise in procedures, observations and conclusions. The details worked out by him are as follows and his color comparisons were made with Ridgway standards (7).

a. Almen test:

To 10 cc. of the bark extract add 2 drops of KOH solution followed by 8 drops of a saturated solution of copper sulfate. Shake thoroughly and then add 8 drops of the Almen reagent. Boil the solution for 10 or 15 minutes. The resulting color is noted 2 to 3 hours later when it has developed maximum intensity and the precipitate which had formed during the reaction has settled to the bottom of the test tube.

Observations

1. Sour orange Cinnamon coloration
2. Rough lemon Pale vinaceous coloration
3. Grapefruit Vinaceous lilac coloration
4. Sweet orange Light rosolane purple

b. Ammonium molybdate test:

To 3 to 4 drops of ammonium molybdate reagent add 5 cc. of the bark extract and boil the solution for 20 to 30 seconds. Within 3 to 5 minutes the reaction gives its most typical color. The tests are then set aside for 24 hours to allow the solutions to clear, thus making it possible to study the results and match the colors.

Observations

1. Sour orange Light sulfate green to cendre green
2. Rough lemon Methyl blue
3. Sweet orange Light sulfate green
4. Sweet orange Cendre green

c. Titanous chloride test:

Two drops of titanous chloride solution are added to 5 cc. of the bark extract and then shaken. The reaction is completed within an hour.

Observations

1. Sour orange Olive green
2. Rough lemon Light brown pink tint
3. Grapefruit Yellow to light brown
4. Sweet orange Vivid green

d. Ferric chloride test:

Add 2 drops of the ferric chloride solution to 5 cc. of the bark extract. Shake at once and observe during the first 15 minutes. The immediate result is indicative of its degree of turbidity and color, with the reaction reaching its peak at the end of this interval.

Observations

1. Sour orange Brown to dark brown, but very clear
2. Rough lemon Pale to straw yellow, cloudy
3. Grapefruit Cream to buff yellow, cloudy
4. Sweet orange Brown, turbid

Varieties of rootstocks under study by Mr. L. C. Masters were

the following:

<u>Sour orange</u>	Standard, African, Brazilian, Paraguay, Seville and Bittersweet
<u>Rough lemon</u>	Rough lemon, Eureka lemon and Lisbon
<u>Grapefruit</u>	All available varieties were used. Regarding this rootstock, the author of the article remarks that "Grapefruit was found to be inconsistent in its reaction".
<u>Sweet orange</u>	Koethan, Madam Vinus, Bessie, Oroville, Indian orchard and Homossasa

The author of this work concludes his findings with remarks to the following effect: "The colors used for the above charts (i.e. with regard to the Almen test and Ammonium molybdate test) were selected, in most cases, from average color reactions of several varieties of each species", and seems to draw the pointed attention of his readers by observing that "these (colors) can be considered only approximate because of the variation in bark samples and the differences in the eyesight of the individuals performing the test".

MATERIALS AND METHODS

Both bark and leaf tissues were studied. The bark used was of two kinds, viz., a) bark from trees of the rootstock varieties which for convenience may be termed as straight bark, and b) bark from a budded tree obtained from below the bud-union. The leaves under test were only from the straight trees and not from the trees budded on top. Bark and leaf samples as above described were taken from each of the four species under test and dried in an oven for at least 24 hours at about 70° C. This temperature is considered optimum by most workers for drying plant tissues. Over-heating may coagulate the proteins, destroy the enzymes and volatilize certain products. If the temperature be lower than the above mark, the drying procedure may become too prolonged thus entailing loss of time and delaying the process unnecessarily. The dried material was run through a mechanical grinder and powdered to a 40-mesh sieve size.

All the reagents were prepared on the lines of the previous workers, details of which are given on page 24, Appendix A.

Samples under study were taken as follows:

1. Sour orange

a. Bark straight: Trees growing on the University Campus

b. Leaves straight: Trees " " " " " "

c. Bark below bud-union: Obtained from Tempe Citrus Farm

1. Sour orange with grapefruit top..... Plot A/19

2. Sour orange with Eureka lemon top Plot D/14

3. Sour orange " " " " Plot D/16

4. Sour orange with Washington navel top Plot F/4

5. Sour orange with " " " " Plot F/5

2. Rough lemon

a. Bark straight: Trees growing on the University Campus

b. Leaves straight: " " " " " " " "

c. Bark from below bud-union with grapefruit top: Obtained from Tempe Citrus Farm, Plot E/3

3. Grapefruit

a. Bark straight: Marsh trees growing on the University Campus

b. Leaves " " " " " " " "

c. Bark from below bud-union: Not available from Tempe or elsewhere

4. Sweet orange

a. Bark straight: Diller and Washington navel trees on the University campus

b. Leaves " " " " " " " "

c. Bark from below bud-union: Obtained from Tempe Citrus Farm

1. Sweet orange with Eureka lemon on top Plot D/12

2. Sweet orange " " " " " " Plot D/13

3. Sweet orange " " " " " " Plot D/17

A sample of .75 grams of the ground dried bark was weighed, placed in a beaker and 20 cc. of distilled water added to it. The suspension was allowed to stand for at least half an hour; and, the contents were agitated occasionally during the interval. The suspension was filtered into a flask, and the residue washed with an additional 10cc. of distilled water. The water extract so obtained was then treated with the

reagents on the same lines as followed by the previous workers.
(For details please refer to (4,5). The color reactions of the
tissues under study are given in the tables Nos. 5 to 8, pages, 18 to
23).

RESULTS AND DISCUSSION

Almen reagent: Color developments with this reagent are given in tables 5 and 6, page 18 to 21. The colors have been described in table 5 in terms of plates outlined in the "Dictionary of Colors" by Maerz and Paul (6), and those in table 6 in terms of "Color Standards and Nomenclature" by Ridgway (7). A reference to these books will show that the main ground-color development in case of each of the rootstock under study is more or less constant throughout; although, with respect to range in shade within that color, they continue to vary slightly from test to test. In case of sour orange, for instance, the main ground-color is, what in common parlance may be described as, "brown" or "dark brown"; for rough lemon it is "brownish pink"; for grapefruit, "smoke transparent" or "almost colorless", and for sweet orange, "pink" or "deep pink". A slight variation in shade within the limit of the main color range noticeable in each test is obviously due to certain laboratory treatment differences that were unavoidable.

In addition to working on bark tests, the writer of this thesis has included the reactions of leaf tissues in his studies. For it has been observed frequently that suckers usually develop from the stock below the bud-union, and should these sucker leaves offer some clue to the root that produced them, an additional facility will naturally become available to a student of rootstock identification. Moreover, leaves provide as compared to bark, "a relatively uniform tissue, that is easily sampled, dried and ground. Also removal of a leaf sample

is much less damaging to the tree than the bark sample⁰⁰ (3).

A series of leaf samples tests run on lines similar to those of bark yielded color reactions usually in agreement with the bark, except for the following differential points:

- a. Leaf reactions were usually a shade deeper in color than those of the bark.
- b. Leaf extract coloration faded away more rapidly than in case of bark. Leaf reaction is, therefore, less stable.

For the sake of making a ready comparison between the findings of the previous workers and the writer of this thesis, the following table showing the average reactions as given by the Almen reagent is presented:

Table 1. Color developments with Almen reagent as reported by various workers

<u>Rootstock</u>	<u>Halma and Haas</u>	<u>C. L. Masters</u>	<u>The writer of this thesis</u>
1. Sour orange	Brown	Cinnamon	Brown
2. Rough lemon	Colorless	Pale Vinaceous	Brownish pink
3. Grapefruit	Light pink	Vinaceous lilac	Smoke transparent or colorless
4. Sweet orange	Pink	Light rosolane purple	Pink or deep pink

A study of the above will show that regarding sour orange reactions, Halma and Haas and the writer of this thesis are in complete agreement, but Masters reports a different color reaction. As regards sweet orange, all three workers are in close agreement, although here Masters uses a rather different phraseology, but in essence he arrives practically at the same conclusion. As for rough lemon and grapefruit, they all

seem to be in marked disagreement on their findings. The writer of this thesis was aware of this fact from the very start and, in order to acquire confidence in his own findings, he has devoted much time and attention to the solution of this discrepancy with the results reported by the previous workers. Accordingly, the test was repeated time and time again, with the materials and reagents renewed often with a view to minimizing, as far as possible, any chance of error or discrepancy. In the light of his own findings the writer is inclined to conclude that the differences in the findings under review are possibly due to variety-differences of stocks used by each worker, combined with variations in soil, climate and cultural factors.

Ammonium molybdate reagent: Findings of the previous workers as well as those of the writer of this thesis are presented below in the form of a chart for a comparative study. (For details reference may be made to table no. 7 on page 22)

Table 2. Color developments with Ammonium molybdate as reported by various workers.

<u>Bark:</u>			<u>Writer of this thesis</u>
<u>Rootstock</u>	<u>Halma and Hass</u>	<u>C. L. Masters</u>	
1. Sour orange	Varying shades of green	Light sulfate to light cendregreen	Cendre green to light cendre green
2. Rough lemon	Deep blue	Methyl blue	Methyl blue to greenish blue
3. Grapefruit	Light blue or bluish gray	Light sulfate green	Light sulfate to sulfate green
4. Sweet orange	Varying shades of green	Cendre green	Cendre green

Leaves:

Table no. 7, page 22 shows that the color reactions of leaf tissues

against this reagent are usually the same, but the color-shade is a bit more intensified.

Scion effect on rootstock:

Table no. 7, page 22, also shows that with ammonium molybdate reagent color reactions on the bark tissue have generally remained constant irrespective of the source of the tissue, i.e., whether it was obtained from a straight stock or from a budded stock. There is, of course, one exception to this. That is, sour orange with Washington navel top yielded sulfate green color instead of a cendre green color, were there no top on this stock.

Titanous chloride and
Ferric chloride reagents:

Both of the above reagents have, oddly enough, failed to give any conclusive reactions, as would appear from the following table:

Table 3. Color developments of Titanous chloride as reported by various workers.

<u>Rootstock</u>	<u>Halma & Haas</u>	<u>C. L. Masters</u>	<u>The writer of this thesis</u>
1. Sour orange	Greenish color	Olive green, clear or cloudy	Olive green, cloudy
2. Rough lemon	V. light pink	Light brown, pink	" " "
3. Grapefruit	Warm gray	Yellow to light brown	Light olive, "
4. Sweet orange	Greenish color	Vivid green	Olive green, "

No definite pink or brown of any shade ever developed with the tests conducted by the writer of this thesis. Different shades of olive

green did appear, but were variable in intensity and character and, as such, almost valueless.

Table 4. Color developments of Ferric chloride reagent as reported by various workers.

<u>Rootstock</u>	<u>Halma & Haas</u>	<u>C. L. Masters</u>	<u>The writer of this thesis</u>
1. Sour orange	Dark clear brown	Brown to dark brown but very clear	Dark brown, turbid
2. Rough lemon	Clear straw yellow	Pale to straw yellow, cloudy	" " "
3. Grapefruit	" " "	Clear to buff yellow, cloudy	" " " (but light shade)
4. Sweet orange	Brownish, turbid	Brown, turbid	Dark brown, turbid

This reagent as well did not prove to be of much practical use as the color reactions showed variations every time the test was repeated and indicated no marked, fixed feature of any importance.

SUMMARY OF RESULTS

1. Tissue coloring tests with chemical reagents for the purpose of identification were conducted on the four commercially important Arizona Citrus rootstocks, viz., Sour orange, Rough lemon, Sweet orange and Grapefruit.

2. Chemical reagents tried were four, viz., Almen reagent, Ammonium molybdate, Titanous chloride and Ferric chloride. For the color tests, the first two reagents have proved very efficacious and the last two of a doubtful utility, at least under Arizona conditions.

3. Almen reagent yields a brown color with sour orange; pink, with sweet orange; pinkish brown or light pink, with rough lemon; and, is transparent or colorless with grapefruit.

4. The ammonium molybdate test helps to segregate rough lemon from sour orange or sweet orange with which it is likely to be confused in the Almen reagent test. Rough lemon gives a methyl blue or occasionally a greenish blue coloration with ammonium molybdate, while sweet orange and sour orange yield a cendre green color and grapefruit gives a light sulfate green to sulfate green.

5. Grapefruit invariably yields a characteristic smoke transparent or almost colorless reaction with the Almen reagent and can seldom be confused with other rootstocks.

6. Leaf tissues of the rootstocks under study give approximately the same colors as the bark tissues; the former, however, usually show a deeper shade within the same color than the latter. The colorations

shown by the leaf tissues seem to fade more readily and appear to be less stable than bark colors.

7. The color development for observation purposes is at its best within the first 24 hours of the reaction. After this period the color starts fading and the sample is no longer reliable.

8. In order to get satisfactory results, it has been found necessary to soak the dried leaf tissue for one-half hour and the bark tissue for two to three hours before filtering. The suspension must be agitated occasionally during the soaking period.

9. To obtain proper color development, it is necessary that the tube containing extract and reagent be boiled over the flame for at least one minute in the case of ammonium molybdate and for two minutes in the case of the Almen reagent.

10. The chemical color tests above described may be further corroborated by studying the characteristic leaf features and bud-union developments as outlined on page 25 of this thesis.

11. To avoid all chance of possible error arising out of any abnormal color behavior due possibly to the peculiar scion-stock effect and other factors, it seems also desirable that first of all, color developments for the locally known rootstocks should be prepared and then the identity of the unknown stocks be determined by making the necessary comparisons of one against the other.

Table 5. Color reactions of straight bark (st. b.) and of straight leaf (st. l.) samples with the Almen's reagent (6).

No. of test	<u>Sour orange</u>		<u>Rough lemon</u>		<u>Grapefruit</u>		<u>Sweet orange</u>									
	st. b.	st. l.	st. b.	st. l.	st. b.	st. l.	st. b.	st. l.								
	plate	range	plate	range	plate	range	plate	range	plate	range	plate	range	plate	range		
1.	5h	D/3-4	55	C/2-3	5h	F/1-2	55	F/2-3	53	A/1-2	5h	A/1-2	5h	I/4-5	5h	I/4-5
2.	5h	F/4-5	55	C/3-4	5h	E/1-2	55	F/1-2	53	B/1-2	5h	A/1-2	5h	F/3-4	5h	I/2-3
3.	5h	F/4-5	55	C/2-3	5h	E/2-3	55	F/2-3	53	B/1-2	5h	A/1-2	5h	G/1-2	5h	I/4-5
4.	5h	F/3-4	55	C/2-3	5h	E/1-2	55	F/2-3	53	B/1-2	5h	A/1-2	5h	F/3-4	5h	I/3-4
5.	5h	E/3-4	55	C/3-4	5h	E/1-2	55	F/2-3	53	A/1-2	5h	A/1-2	5h	F/2-3	5h	I/4-5
6.	5h	E/3-4	55	E/4-5	5h	E/4-5	55	F/2-3	53	B/1-2	5h	B/1-2	5h	G/4-5	5h	I/4-5
7.	5h	E/2-3	55	E/4-5	5h	E/3-4	55	F/1-2	53	A/1-2	5h	A/3-2	5h	F/2-3	5h	H/2-3
8.	5h	F/2-3	55	C/2-3	5h	E/4-5	53	F/3-4	53	A/1-2	5h	A/1-2	5h	G/3-4	5h	H/3-4
9.	5h	F/5-6	55	E/2-3	5h	F/1-2	53	F/2-3	53	A/1-2	5h	A/1-2	5h	F/3-4	5h	I/1-2
10.	5h	F/4-5	55	E/3-4	5h	F/2-3	55	F/1-2	53	A/1-2	5h	A/1-2	5h	F/2-3	5h	I/3-4
11.	5h	D/2-3	55	C/3-4	5h	E/3-4	55	F/2-3	53	A/1-2	5h	A/1-2	5h	F/3-4	5h	I/3-4
12.	5h	D/2-3	55	C/4-5	5h	E/4-5	55	F/2-3	53	A/1-2	5h	A/1-2	5h	F/2-3	5h	I/3-4
13.	5h	E/3-4	55	C/2-3	5h	E/2-3	55	F/1-2	53	A/1-2	5h	B/1-2	5h	G/2-3	5h	I/1-2
14.	5h	E/3-4	55	E/4-5	5h	F/2-3	53	F/3-4	53	A/1-2	5h	B/1-2	5h	G/3-4	5h	I/4-5
15.	5h	E/2-3	55	E/3-4	5h	F/1-2	53	F/3-4	53	B/1-2	5h	B/1-2	5h	G/3-4	5h	I/4-5
16.	5h	F/5-6	55	C/3-4	5h	E/4-5	53	F/1-2	53	B/1-2	5h	B/1-2	5h	G/3-4	5h	I/4-5

(continued on the next page)

3. The plate and range of colors are quoted from the "Dictionary of Colors" by Maerz and Paul-- 1st. edition, 1930.

Table 5 (cont.)

No. of test	Sour orange		Rough lemon		Grapefruit		Sweet orange	
	st. b.	st. l.	st. b.	st. l.	st. b.	st. l.	st. b.	st. l.
	plate range	plate range	plate range	plate range	plate range	plate range	plate range	plate range
17.	54 F/5-6	55 C/2-3	54 F/2-3	55 F/1-2	53 B/1-2	54 A/1-2	54 F/2-3	54 I/4-5
18.	54 D/2-3	55 C/3-4	54 F/2-3	53 F/1-2	53 A/1-2	54 B/1-2	54 F/1-2	54 I/3-4
19.	54 F/5-6	55 C/3-4	54 F/1-2	53 F/1-2	53 A/1-2	54 A/1-2	54 F/1-2	54 H/3-4
20.	54 F/3-4	55 C/2-3	54 F/2-3	53 F/1-2	53 A/1-2	54 B/1-2	54 F/3-4	54 H/3-4
21.	54 F/4-5	55 C/3-4	54 F/2-3	53 F/2-3	53 A/1-2	54 B/1-2	54 G/3-4	54 I/4-5
22.	54 D/1-2	55 E/1-2	54 F/3-4	55 F/2-3	53 A/1-2	54 B/1-2	54 G/4-5	54 I/4-5
23.	54 F/3-4	55 E/1-2	54 F/3-4	55 F/1-2	53 A/1-2	54 A/1-2	54 G/1-2	54 I/2-3
24.	54 F/1-2	55 E/1-2	54 F/3-4	55 F/2-3	53 A/1-2	54 A/1-2	54 G/3-4	54 H/4-5
25.	54 D/1-2	55 C/3-4	55 F/2-3	53 F/3-4	53 A/1-2	54 A/1-2	54 F/1-2	54 I/2-3
26.	54 D/3-4	55 E/3-4	55 F/2-3	55 F/2-3	53 A/1-2	54 A/1-2	54 F/3-4	54 I/2-3
27.	54 F/3-4	55 C/4-5	54 E/4-5	55 F/1-2	53 B/1-2	54 B/1-2	54 F/3-4	54 I/4-5
28.	54 E/2-3	55 C/4-5	54 E/3-4	55 F/3-4	53 A/1-2	54 A/1-2	54 F/1-2	54 I/2-3
29.	54 C/1-2	55 E/4-5	54 E/3-4	55 F/1-2	53 B/1-2	54 B/1-2	54 F/4-5	54 I/2-3
30.	54 D/2-3	55 E/2-3	54 F/2-3	53 F/3-4	53 A/1-2	54 A/1-2	54 H/3-4	54 I/2-3
31.	54 D/2-3	55 E/1-2	54 E/2-3	55 F/3-4	53 B/1-2	54 A/1-2	54 F/2-3	54 I/2-3
32.	54 D/3-4	55 E/4-5	54 F/2-3	55 F/3-4	53 A/1-2	54 A/1-2	54 F/1-2	54 I/2-3
33.	54 D/3-4	55 E/4-5	54 F/2-3	55 F/1-2	53 A/1-2	54 B/1-2	54 F/3-4	54 I/2-3

(continued on the next page)

Table 5 (cont.)

No. of test	<u>Sour orange</u>		<u>Rough lemon</u>		<u>Grapefruit</u>		<u>Sweet orange</u>	
	st. b.	st. l.	st. b.	st. l.	st. b.	st. l.	st. b.	st. l.
	<u>plate range</u>	<u>plate range</u>	<u>plate range</u>	<u>plate range</u>	<u>plate range</u>	<u>plate range</u>	<u>plate range</u>	<u>plate range</u>
34.	54 D/1-2	55 F/1-2	54 F/2-3	53 F/1-2	53 A/1-2	54 B/1-2	54 F/3-4	54 I/2-3
35.	54 F/3-4	55 C/3-4	54 F/2-3	53 F/1-2	53 A/1-2	54 A/1-2	54 E/4-5	54 I/2-3
36.	53 E/1-2	55 C/3-4	54 F/4-5	53 F/1-2	53 A/1-2	54 A/1-2		
37.	54 F/2-3	55 C/3-4	54 E/2-3	53 F/1-2	53 B/1-2	54 B/1-2	54 E/2-3	54 I/2-3
38.	54 E/1-2	55 C/1-2	54 E/3-4	55 F/3-4	53 A/1-2	54 B/1-2	54 F/2-3	54 H/2-3
39.	54 E/2-3	55 E/3-4	54 F/1-2	55 F/1-2	53 B/1-2	54 B/1-2	54 F/4-5	54 I/2-3
40.	54 E/3-4	55 E/1-2	54 F/1-2	55 F/1-2	53 B/1-2	54 B/1-2	54 F/4-5	54 I/1-2
41.	54 E/3-4	55 E/2-3	54 F/1-2	55 F/1-2	53 B/1-2	54 A/1-2	54 F/1-2	54 I/2-3
42.	54 E/3-4	55 E/4-5	54 F/2-3	55 E/3-4	53 A/1-2	54 A/1-2	54 F/2-3	54 I/2-3
43.	54 E/3-4	55 E/4-5	54 F/2-3	55 F/1-2	53 B/1-2	54 A/1-2	54 F/2-3	54 I/2-3
44.	54 E/3-4	55 E/4-5	54 E/2-3	55 E/1-2	53 A/1-2	54 A/1-2	54 F/4-5	54 I/1-2

Table 6. Color reactions of different rootstocks with Almen reagent according to Ridgway Standards (7).

(Specimen No. 1 to 12 obtained from University Citrus Farm at Tempe)

Serial No.	Plot No.	Rootstock with nature of top	Color Description with Ridgway St'ds.	Plate No.	Remarks
1.	D/4	Sour orange (E.L. top)	Livid brown	38	Bark below the bud-union.
2.	F/3	" " (W.N. top)	Vetnonia purple	38	"
3.	G/1	" " (Valencia top)	" "	38	"
4.	D/16	" " (E.L. top)	Light brownish drab	45	"
5.	F/4	" " (W.N. top)	Vetnonia purple	38	"
6.	G/3	" " (Valencia top)	Brownish drab	45	"
7.	G/5	" " " "	Deep brownish drab	45	"
8.	F/5	" " (W.N. top)	" "	45	"
9.	D/12	Sweet orange (E.L. top)	Dephne red	38	"
10.	D/13	" " " "	" "	38	"
11.	D/17	" " " "	" "	38	"
12.	E/3	Rough lemon (G.F. top)	Russet vinaceous	39	"
13.	Local	Sour orange	Dark livid brown	39	Straight bark used
14.	"	Sweet orange	Livid brown	39	" " "
15.	"	Grapefruit	Smoke gray	46	" " "
16.	"	Rough lemon	Light russet vinaceous	39	" " "
17.	"	Sour orange	Dephne red	38	Straight-leaves
18.	"	Sweet orange	Rocellin purple	38	" " "
19.	"	Grapefruit	Pale acru drab	46	" " "
20.	"	Rough lemon	Dephne red	38	" " "

Table 7. Color Reactions of the Citrus Rootstocks tissues with Ammonium molybdate reagent

(According to Ridgway Standards (7))

Serial No.	Description of the rootstock	Color Reaction	Remarks
1.	Sour orange (E.L.top)	cendre green	Bark obtained from Tempe citrus farm
2.	" " (W.N.top)	sulfate green	"
3.	" " (Valencia top)	cendre green.	"
4.	" " (E.L.top)	" "	"
5.	" " (W.N.top)	" "	"
6.	" " (Valencia top)	" "	"
7.	" " " "	" "	"
8.	" " (W.N.top)	" "	"
9.	Sweet orange (E.L.top)	" "	"
10.	" " " "	" "	"
11.	" " " "	" "	"
12.	Rough lemon (G.F.top)	greenish blue	"
13.	Sour orange	light cendre green to cendre green	Straight bark obtained locally
14.	Rough lemon	deep greenish blue to methyl blue	"
15.	Grapefruit	light sulfate green to sulfate green	"
16.	Sweet orange	cendre green	"
17.	Sour orange leaves	cendre green	Straight leaves obtained locally
18.	Rough lemon leaves	deep bluish green	"
19.	Grapefruit leaves	sulfate green to deep cendre green	"
20.	Sweet orange leaves	cendre green to deep cendre green	"

Table 8. Color reactions of the citrus rootstocks tissues by (a) titanous chloride and (b) ferric chloride.

a. Titanous chloride reagent

<u>Bark</u>	<u>Remarks</u>
1. Sour orange -- olive green, cloudy	Tests were repeated many times to arrive at some conclusive findings, but no definite reaction was available, except very slight difference in color shade.
2. Rough lemon -- " " "	
3. Grapefruit -- light olive green, cloudy	
4. Sweet orange -- olive green, cloudy	

b. Ferric chloride

<u>Bark</u>	<u>Remarks</u>
1. Sour orange -- dark brown	The above remarks hold equally good
2. Rough lemon -- " "	
3. Grapefruit -- " " (slightly lighter)	
4. Sweet orange -- " "	

Appendix (A) List and method of preparation of the various reagents used in the citrus rootstock identification by the Tissue Coloring Test

1. Almen reagent: Weigh out 110 grams of pure mercury in a beaker and 110 cc. of red fuming nitric acid C.P. (sp.gr. 1.60). A slight shaking will start at once the chemical action accompanied by clouds of thick red fumes. Place the beaker in a hood or use any other effective device to keep the fumes from poisoning the atmosphere. In about an hour or so, the chemical action will be complete and the fumes will cease. Dilute the resulting liquid by adding to it 220 cc. of distilled water. The Almen reagent is now ready for use.

2. Ammonium molybdate: Dissolve 100 grams of molybdic acid in a mixture of 114 cc. of ammonium hydroxide (sp.gr. 0.90) and 271 cc. of distilled water. Pour slowly with a constant shaking into a COOL mixture of 489 cc. of nitric acid C.P. (sp. gr. 1.42) and 1148 cc. of distilled water.

3. Titanous chloride: This is used as a 20% solution in distilled water and is obtainable in the ready made form from chemical manufacturers.

4. Ferric chloride: Is a saturated solution of C.P. FeCl_3 in distilled water. (125 grams of FeCl_3 in 29 cc. of H_2O).

Appendix (B) Identification of Rootstocks for oranges by methods other than chemical tests.

According to W. P. Bitters (1), the above described chemical color tests of rootstock identification are "reliable for sweet orange stock and sour orange stock; the reliability of the method diminishes for other types of stocks. Variables in the age of the tree, thickness of the bark, the nearness of the samples to the bud-union, the scion variety, the freedom or the presence of disease, the presence of surface contaminants, et cetera, are all factors which may affect the tests and render identification uncertain".

In view of the above facts, it would not be overemphasizing the value and usefulness of getting at some morphological or physical features of these rootstocks which could be utilized as a means to verify and to corroborate the findings arrived at by the aforementioned color tests. Fortunately, however, foliage characters and the types of bud-unions of the commonly used rootstocks have features of their own and an intelligent use of these will, no doubt, greatly help solve the problem of rootstock identification. Below are given some of the more important characteristics of these stocks that may be of practical utility.

Foliage characters:

1. Sweet orange leaves: They are medium in size, pointed at the top and rather rounded at the base. The petiole is usually winged, narrow and does not overlap the leaf blade. Moreover, these leaves have

petioles that are only about one-third in size as compared to that of the sour orange leaf, and for this reason there should be no confusion between the two. Crushed leaves of this stock emit no marked aroma either.

2. Sour orange leaves: Here leaves are usually larger than those of the sweet orange. Petioles are also relatively longer and more broadly winged. The tip of the leaf is often pointed and the base is usually wedge-shaped. When crushed, the leaves yield a peculiar pungent sort of aroma.

3. Grapefruit leaves: They are larger in size than either sweet or sour orange, are slightly oval in shape, rather blunt at the tip and rounded rather than wedge-shaped at the base. The petiole usually terminates with broad wings which more frequently touch or overlap the leaf-base.

4. Rough lemon leaves: These have rather an oval form terminating in a blunt tip. They are relatively of light shade green color and are characterized by absence of any wings at the leaf-base. When held up to light, the leaves reveal placements of conspicuous oil glands.

Bud-Union Features:

A study of the scion-stock combination at the point of the bud-union frequently offers an added help in the rootstock identification. This study will, of course, be more dependable in cases where stocks are budded high and planted high in the orchard. If placed low, identification of the bud-union may cause considerable confusion as the roots would start close to the soil surface and consequently may not reveal

the normal differences in the shape of the bud-union that would be noticeable otherwise.

According to their form of combinations and eventual development, the scion-stock unions may be classified into three types as follows:

(a) Smooth bud-union: This union results usually from a sweet orange top on a sweet orange or rough lemon rootstock. In this case, girth both of the stock and the scion is about equal. Bark of the stock is smooth, contours of the union, if any, are also smooth, and the color of the bark above and below the union is usually identical.

(b) Undergrowth of the stock: This would hold true when sweet orange is budded on a sour orange stock. In such a case, the undergrowth of the stock is usually quite noticeable, but variations associated with different strains within the same rootstock are likely to occur. There are, however, no distinguishable features in the bark characteristics of the stock and the scion.

(c) Overgrowth of the stock: This type of bud-union is frequently met with where grapefruit is used as a stock with sweet orange budded at the top. Here the stock portion displays an overgrowth relative to the scion portion giving to the former a sort of bulging effect. This enlarged base feature in grapefruit stock renders it readily distinguishable from sweet orange or rough lemon stocks.

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