CHARACTERIZATION OF CITRUS CULTIVARS AND SEPARATION OF NUCELLAR AND ZYGOTIC SEEDLINGS BY THIN LAYER CHROMATOGRAPHY

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Abstract. **The flavonoids and coumarins of several citrus cultivars were studied and a simple thin-layer chromatographic (TLC) method was developed to distinguish nucellar from zygotic seedlings in a breeding population. Leaf extracts were prepared from young and mature plants and were examined by TLC with various solvent systems. Each species gave a distinctive TLC pattern and contained characteristic "marker" compounds. Zygotic plants usually resulted in TLC patterns that lacked the marker compounds, showed much lower concentration than the parents, contained markers from both parents, and some contained compounds not found in either parent. TLC patterns of nucellar seedlings were identical to that of the seed parent.**

There is a need for new citrus cultivars resistant to disease, insects, and climatic extremes, which produce fruit with high quality and good flavor. Earlier maturing citrus would permit more efficient use of harvesting labor, packinghouses and processing equipment. The biggest problem in the use of citrus seed parents that produce a mixture of nucellar and zygotic embryos is the recognition of the young zygotic seedlings, because the vegetative characteristics of orange cultivars are very similar (8). A simple chemical test that could distinguish zygotic from nucellar seedlings would increase the rate of development of new commercial cultivars by avoiding the necessity of waiting until fruit is formed (5-8 years) before they can be distinguished.

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Reference to specific commercial products does not constitute endorsement.

Little progress has been made in hybridization of sweet orange *(Citrus sinensis)* varieties because no sweet orange seed parent that produces many zygotic seedlings has been available (4). Most of the zygotic seedlings that have been obtained from the use of sweet orange seed parents have occurred when *Poncirus trifoliata* was the pollen parent. In these cases the occasional zygotic seedlings can be recognized readily by the dominant trifoliate leaf character.

Frost (3) reported that the few 'Ruby' X 'Valencia' orange hybrids that he obtained were nearly all inferior in vigor and conspicuously weak. He cited several reports that extremely heterozygous citrus forms, including the sweet orange, produce zygotic seedlings with widely varying characters. Certain characters may even be outside the parental range.

A number of chemical tests have been tried for identifying zygotic versus nucellar seedlings at an early stage. Furr et al. (6) and Nishiura et al. (12) used modifications of the rootstock color reaction test, Pieringer and Edwards (13) used infrared spectroscopy and Pieringer et al. (14) tried gas chromatography as a basis of distinction. None of these tests have proven to be entirely satisfactory. Kefford (10) and Horowitz (9) were the first to correlate specific flavonoid compounds with certain taxa of citrus. Albach and Redman (1) related flavanone compositions to inheritance, while Stanley and Jurd (15) reviewed the coumarins and psoralens found in citrus. Tatum and Berry (16) studied the methoxy flavonoids found in Valencia orange *(C. sinensis)* and Robinson tangerine [(*C. reticulata* x *C. paradisi* x *C. reticulata*)] while Nagy and Nordby (11) have recently shown that four citrus species—*C. sinensis*, *C. paradisi*, *C. reticulata* and *C. limon*—have distinctive lipid profiles. The purpose of this study was to develop a simple thin-layer chromatography (TLC) method to distinguish zygotic from nucellar orange seedlings, based upon the previous work of Albach (1), Stanley (15) and Tatum (16).

Materials and Methods

Production of zygotic seedlings

The 'Mediterranean Sweet' orange (*Citrus sinensis* [L.] Osbeck) reported by Hearn (7, 8)

was used as the seed parent in a cross with 'Pineapple' orange *(C. sinensis)* in the spring of 1971. At the same time, this seed parent was pollinated with 'Argentina' trifoliate orange (*Poncirus trifoliata* [L.] Raf.). All flowers, except those that were mature but unopened, were removed from the branches to be pollinated. The petals and stamens were removed and the stigmas immediately were thoroughly covered with fresh pollen. They were not covered after pollination since bees rarely visit citrus flowers from which the petals and stamens have been removed (Furr [5] and confirmed by the coauthor).

The zygotic seedlings from 'Mediterranean Sweet' x 'Argentina' could be recognized by their trifoliate leaves. Thus, bonafide nucellar seedlings of 'Mediterranean Sweet' were obtained. These nucellar seedlings, seedlings from 'Mediterranean Sweet' x 'Pineapple' produced in 1971 and the parent trees were included in the study.

Samples

Leaves of various citrus trees were obtained from Whitmore Foundation Farm (U. S. Horticultural Research Laboratory, USDA, Orlando, Florida). Leaves of the 'Clementine' mandarin were obtained from the Agricultural Research and Education Center (Lake Alfred, Florida).

Preparation of leaf extract

Light green, fully expanded, immature leaves were collected, washed with water, and air dried. The samples were weighed (2-6 gm), cut with scissors into approximately 1-cm widths and placed into a Waring Blender (Model 1042). Sixty ml of methanol was added to the leaves and blended for one min at low speed. A 10 ml aliquot was removed for TLC analysis. The sample remaining in the blender was filtered and saved. All sweet orange samples were combined, and sinensetin, the main marker in sweet orange was isolated from these extracts (16).

Leaf samples were obtained from nine sweet oranges: *(C. sinensis)* cv. 'Pineapple', 'Pope Summer Sweet', 'Mediterranean Sweet', four nucellar seedlings from the 'Mediterranean Sweet', 'Hamlin', 'Shamouti', 'Lue Gim Gong', 'Valencia', 'Parson Brown' and 'Sanford Mediterranean Sweet'; 'Palestine Sour' (*C. aurantium* L.), 'Australian Sour' *(C. aurantium hybrid)*, 16 hybrids from 'China pummelo' (*C. grandis* [L.] Osbeck) X 'Pineapple' orange; 2 mandarins *(C. reticulata)* cv. 'Clementine' and 'Dancy'; 11 mandarin hybrids: 'Orlando', 'Minneola', 'Seminole', and 'Sampson' tangelos (*C. paradisi* X *C. reticulata*); Robinson', 'Osceola', 'Nova' and 'Lee' ('Clementine' X 'Orlando'); 'Page' ('Minneola' X 'Clementine'); 'Wekiwa' ('Sampson' X 'Duncan' grapefruit (*C. paradisi* Macf.); and 'Murcott' (*C. reticulata* hybrid); 'Marsh' and 'Duncan' grapefruit *(C. paradisi)*; 3 presumed grapefruit hybrids: 'Triump', 'Royal' and 'Mott'; 1 lime *(C. aurantifolia)* cv. 'West Indian' or 'Key' and the 'Rangpur lime' (*C. reticulata* var. *austera* Swing.), and 50 seedlings from 'Mediterranean Sweet' X 'Pineapple' oranges.

Thin-layer chromatography

Plates were of Silica Gel GF (20 x 20 cm, 250 μ, Analtech, Inc., Wilmington, Delaware) and Baker-flex Polyamide 6 (20 x 20 cm, J. T. Baker Chemical Co., Phillipsburg, New Jersey). Solvent systems were (A) chloroform-acetic acid, 99-1 by volume; (B) benzene-acetone-acetic acid, 43-5-2; (C) hexane-benzene-acetone-methanol, 6-3-1-0.5, (D) hexane-benzene-acetone-methanol, 6-3-1-0.05; (E) benzene-acetic acid-water-nitromethane, 34-32-5-18; and (F) nitromethane-methanol, 5-2. Solvent systems A-E were used with the Silica Gel GF plates and F was used with the Polyamide plates. Solvent systems A-D were used for the "non-polar" portion of the leaf extract. Solvent systems E and F were used on the polar portion of the leaf extract (which contained the flavanone glycosides [1]).

All TLC tanks were paper lined, and equilibrated. The plates were prepared with 20 channels by using a TLC plate scriber, and leaf extracts were applied quantitatively to the TLC plates with a 50 μl syringe. When Silica Gel GF plates were used, the amount of extract applied represents the extract from 0.5 mg, 1 mg or 1.5 mg of leaves, for Polyamide plates 0.5 mg.

Two spray reagents were used for visualization: (1) 10% sulfuric acid in ethanol, heat 10 min. at 150°C, (2) 1% $AlCl_3$ in methanol. When spray 1 was used, the plate was backlighted with long wave UV light and photographed. When spray 2 was used, the plate was illuminated from above with long wave UV light.

Color transparencies of plates were made with Kodak high speed Ektachrome B (EHB-135) film. Three Kodak gelatin filters (2A, 4 and 15) were used. The 2A enhanced the blue and black, the 4 enhanced the red and orange and the 15 enhanced the yellow. Filters 2A, 4 and 15 were used on solvents A-D, but only filter 15 was satisfactory on solvents E and F. The best exposure time was 16 sec at f-22.

Spectrophotometric methods

For identification of sinensetin the infrared

spectra was run on KBr pellets on a Perkin Elmer 137 spectrophotometer and mass spectra were determined on a DuPont Model 21-490 Mass Spectrometer. The other methoxy flavonoids were identified by TLC Rf, and color under UV light.

Results and Discussion

Light green immature leaves were used in these studies, because mature leaves contain large amounts of chlorophyll which sometimes interfere with the analyses. When mature and immature leaf samples were obtained from two 'Valencia' trees, the mature samples gave identical TLC patterns, as did the immature samples. The difference in the mature and immature leaf samples was mainly in the concentration of the components present.

Since a reliable procedure had not been developed to distinguish zygotic seedlings, our first work was with known hybrids and their parents. When leaf extracts from 13 mandarin or mandarin hybrids were examined as described in the methods section, the mandarins were found to contain two or three "marker" compounds which were tentatively identified as methoxy flavonoids: tangeretin, nobiletin and sinensetin. The concentration of these components varies among cultivars. The cross of 'Clementine' x 'Orlando' gave 'Nova', 'Osceola', 'Robinson' and 'Lee'. The marker compounds in three out of four of these showed concentration intermediate between the two parents. Some of these mandarins were part grapefruit but lacked the "markers" found in grapefruit. Some only contained two of the "markers."

When 'China' pummelo and the two grapefruit samples were examined, each of their nonpolar fractions contained the identical three "markers" tentatively identified as coumarins. 'Mott', 'Royal' and 'Triumph' (grapefruit hybrids) gave coumarin patterns identical to grapefruit, but their polar components were different from that of grapefruit. Their naringin content was very low and they contained neohesperidin in high concentration. The TLC patterns of 'Mott', 'Royal' and 'Triumph' were undistinguishable. When the 16 'China' pummelo x 'Pineapple' orange progeny were examined, the nonpolar TLC patterns showed that all 16 of these hybrids contained the coumarins found in the 'China' pummelo. In five of these the coumarin concentration was extremely low, six showed no variation on the TLC plate and five showed minor variations in concentration. In the polar TLC patterns seven were identical to the 'China' pummelo. Two of these had very low naringin content, two lacked naringin and contained hesperidin, two lacked naringin and one lacked naringin and contained an unknown flavanone. Two showed minor variations in the flavanone patterns from that of the 'China' pummelo. When the data from the polar and nonpolar fractions were combined, 11 hybrids had positive differences. Three of the 16 showed no variation. Two had minor differences. Since these trees all have borne fruit which is different from either parent and were proven hybrids, this TLC test was 81% accurate in distinguishing them.

Other studies were made to determine applicability of the method to sour orange, and lime crosses. 'Palestine' and 'Australian' orange samples both contained neohesperidin. The 'Australian' sour contained coumarins while the other did not and they both apparently lacked the previously found orange "marker." The "Australian' sour orange, 'Key' lime and the 'Rangpur' contained coumarins but they were not identical and each had a distinctive TLC pattern. The 'Rangpur' lime is thought to be a cross between a lime and a mandarin. It contains a large spot with an Rf that corresponds to tangeretin which is a "marker" found in mandarins.

When nine sweet orange cultivars and the four nucellar seedlings from the 'Mediterranean Sweet', were examined with polar and nonpolar solvents, their TLC patterns were found to be almost identical. The main "marker" compound in the nonpolar fraction was sinensetin, and in the polar fraction the "marker" was hesperidin. The 'Hamlin' orange sample had a low sinensetin concentration and might be a hybrid but this has not been proven.

These results established the fact that distinctive TLC patterns could be obtained for various species. Hybrid plants usually resulted in TLC patterns that lacked the parents' "marker" compounds, showed much lower concentartion than the parents, or contained the "markers" from both parents and some contained compounds not found in either parent.

Fifty seedlings of a 'Mediterranean Sweet' orange x 'Pineapple' orange were examined by TLC. Hearn (7, 8) reported that this selection of 'Mediterranean Sweet' orange produced 32% monoembroyonic seed and, then 62% zygotic seedlings when *Poncirus trifoliata* pollen was used. Other than the significant quantities of monoembryonic seed and zygotic seedlings produced when crossed with *P. trifoliata*, vegetative characters of this orange selection are essentially indistinguishable

from other midseason oranges. The fact that zygotic seedlings can be recognized (by vegetative character) among the progeny of 'Mediterranean Sweet' x 'Pineapple' has not been reported previously.

Vegetative characters among the zygotic seedlings are highly variable, indicating that these orange parents, although similar in most characters, are extremely heterozygous as Frost (3) speculated. Although many orange characters are evident in the zygotic seedlings, some show leaf petiole and blade characters that are considered typical of other species of *Citrus*. This finding would be expected in view of the report by Frost that the sweet orange may be of hybrid origin and that differentiation of varieties may have occurred largely by somatic mutation.

Some of the characters of the zygotic seedlings that are suggestive of other species include leaf and twig characters of: pummelo *(C. grandis)*, grapefruit *(C. paradisi)*, mandarin *(C. reticulata)*, sour orange *(C. aurantium)*, and lime *(C. auranti-*

Table 1. 'Mediterranean Sweet' Orange x 'Pineapple' Orange Seedlings. Block 1, Foundation Farm, by J. Hearn.

	Plant	Description - Morphological
1.	11-6	Foliage and leaves show mandarin characters.
2.	11-8	Foliage and leaves show mandarin characters, or may be a triploid, a few cupped leaves could indicate grapefruit characters.
3.	11-15	Foliage and twigs show mandarin characters.
4.	11-25	Foliage and twigs show lime or lemon characters.
5.	11-30	Leaf petioles suggest grapefruit characters.
6.	11-38	Slight indication of mandarin characters.
7.	11-40	Slight indication of mandarin characters.
8.	11-50	Typical sweet orange characters (but slight suggestion of zygotic).
9.	11-53	Typical sweet orange characters (but slight suggestion of zygotic).
10.	11-89	Winged petioles suggest grapefruit characters but small leaved.
11.	11-103	Cupped leaves suggest grapefruit, but may be a triploid.
12.	11-105	Predominately orange characters but slightly different from orange.
13.	11-107	Wide petioles suggest pummelo characters.
14.	11-114	Leaf and twig characters suggests mandarin or sour orange.
15.	12-5	May be sweet orange but slight suggestion of mandarin characters.
16.	12-35	Indication of grapefruit characters but may be a triploid.
17.	12-38	Indications of lime characters.
18.	12-48	Leaf and twig characters suggest mandarin (could possibly be an orange).
19.	12-86	Zygotic, but only orange characters - may be a triploid.
20.	12-102	Petiole, leaf and twig characters suggest pummelo.
21.	12-111	Large leaves and petioles show pummelo characters.
22.	12-112	Predominately orange but slight indication of mandarin characters.
23.	12-121	Petioles suggest pummelo character.

folia) or lemon *(C. limon)*. The leaf characters, other than orange, that appear most commonly among the zygotic seedlings are suggestive of mandarin or the species with a winged petiole (pummelo or grapefruit).

Hearn examined the fifty seedlings prior to chemical analyses and wrote a short morphological description for these seedlings. This description is shown in Table 1 for the seedlings he thought were zygotic. Twenty-seven of the seedlings he described possessed typical sweet orange characters and he stated they were probably nucellar. When the TLC patterns of these 27 seedlings were compared to nucellar seedlings they were found to be identical.

Table 2 describes the chemical differences found in 16 of the 23 plants shown in Table 1. Hearn was able to distinguish 43 out of 50 seedlings or 86%, by observation of morphological characteristics. Thus, it was shown possible to distinguish these by visual observation but this is a rarely found ability. In the 7 plants in Table 2, that appeared to be nucellar by chemical analysis, there may be some zygotic seedlings. This also applies to the 27 plants that were presumed nucellar. This cannot be confirmed until fruit is formed.

As shown in Table 2, some of these seedlings show chemical components (coumarins, naringin and neohesperidin) not found in the parents. It is possible that all of the methoxy flavonoids are present in the parents (16) but if so they are present at extremely low concentration. Samples 13, 17, 20 and 21 contained the same coumarins found in

Table 2. 'Mediterranean Sweet' Orange x 'Pineapple' Orange Seedlings. Block 1, Foundation Farm.

	Plant	Components Different From Nucellar Seedlings
1.	11-6	Sinensetin weak.
2.	11-8	Unidentified flavanone - lacks hesperidin.
3.	11-15	Unidentified flavanone - lacks hesperidin - lacks sinensetin.
4.	11-25	Sinensetin, weak - nobiletin
5.	11-30	Sinensetin, weak - lacks hesperidin - naringin, weak.
6.	11-38	*
7.	11-40	Sinensetin, weak - nobiletin
8.	11-50	*
9.	11-53	*
10.	11-89	Sinensetin, weak - neohesperidin
11.	11-103	Lacks sinensetin
12.	11-105	Sinensetin - nobiletin - tangeretin mandarin pattern
13.	11-107	Lacks sinensetin - lacks hesperidin - coumarins - naringin.
14.	11-114	Lacks sinensetin - lacks hesperidin.
15.	12-5	*
16.	12-35	Sinensetin, weak - neohesperidin.
17.	12-38	Lacks sinensetin - lacks hesperidin - coumarins - naringin.
18.	12-48	*
19.	12-86	*
20.	12-102	Sinensetin, weak - neohesperidin - coumarins - naringin.
21.	12-111	Lacks sinensetin - lacks hesperidin - coumarins - naringin.
22.	12-112	Lacks hesperidin.
23.	12-121	*

*Appears to be nucellar.

pummelo and grapefruit but the concentration varied for all four plants. It is apparent that these plants will produce fruit different from the parents. Since the flowers were not covered following pollination, the possibility of a rare insect visit to an emasculated and pollinated flower cannot be ruled out. However, Cameron and Frost (2) indicated that characters of citrus progeny may even be outside the parental range due to heterozygosity. The only description of orange x orange hybrids is that they are conspicuously weak. Few have been produced and no description of foliage and twig characters could be found. Since a wide range of progeny characters would be expected, we cannot be certain whether the existence in a few cases of certain vegetative and chemical characters not evident in the parents resulted from pollen contamination by insects or transgressive segregation.

Among the compounds shown in Table 2, only the sinensetin was positively identified, the others were tentatively identified by TLC Rf's and colors under UV before and after sprays 1 and 2 (see Table 3) when compared with "knowns." This does not constitute adequate chemical proof to unequivocally establish the presence of each component in the plant material. The TLC patterns showed positively, that the plants were different and that the observed differences were reproducible. This was primarily the intent of the current study.

Color photographic transparencies could be compiled for various citrus species. Since they record specific TLC patterns accurately they should be a great aid in citrus taxonomy. When color slides of all the TLC plates were compared, the nonpolar plates resulted in excellent color reproduction and permanent records. The photographic filters enhanced the various colors and in many cases concentration variations could be detected that were not distinguishable by eye under UV light. Also, some differences were observed on the transparencies that had been overlooked when the plates were examined by eye.

When we examined the 9 varieties of sweet orange only 6 different solvent systems were used. Minor variations were observed but were not definitive. It may be possible to distinguish between these with other solvent systems and spray reagents. Further work is planned along this line. Also, there are other compounds in mandarins that could be used as "markers." Probably there are additional "markers" in the other species as well, and these possibilities are under study.

In conclusion, a TLC analytical procedure has been developed which can be used to distinguish zygotic from nucellar seedlings. Only two or three leaves are required to test each plant. It is relatively fast and at least twenty samples could be checked in a day. This test could be a great help to development of breeding programs.

Table 3. Rf's and Colors of "Marker" Compounds Found in Orange, Mandarin and Grapefruit.

	Rf/19 cm	Color/UV*
Sinensetin	0.26 B, 0.21A	bright yellow
Nobiletin	0.32 B, 0.23A	yellow/brown
Tangeretin	0.36 B	black
Coumarin	0.06A	blue
Coumarin	0.45 B, 0.32A	blue/white
Coumarin	0.59 B, 0.53A	blue

A,B = solvent systems
*After spray 1

Literature Cited

1. Albach, R. F. and G. H. Redman. 1969. Composition and inheritance of flavanones in citrus fruit. *Phytochem.* 8:127-143.
2. Cameron, J. W. and H. B. Frost. 1968. Genetics, breeding and nucellar embryony. In W. Reuther, L. D. Batchelor and H. J. Webber "The Citrus Industry," Vol. II:333-335.
3. Frost, H. B. 1943. Genetics and breeding. In H. J. Webber and L. D. Batchelor, "The Citrus Industry" Vol. I:817-913.
4. Furr, J. R. 1969. Citrus breeding for the arid Southwestern United States. *Proc. Int'l. Cit. Symp.* 1:191-197.
5. Furr, J. R., J. B. Carpenter and A. A. Hewitt. 1963. Breeding new varieties of citrus fruits and rootstocks for the Southwest. *J. Rio Grande Valley Hort. Soc.* 17:90-107.
6. Furr, J. R., P. C. Reece, and G. Hrnciar. 1946. Identification of hybrid and nucellar citrus seedlings by a modification of the rootstock color test. *Proc. Fla. State Hort. Soc.* 59:38-42.
7. Hearn, C. J. 1973. Recently released cultivars and the development of new hybrids of citrus fruits in Florida. Proc. First International Congress of Citriculture, Murcia, Spain—in press.
8. Hearn C. J. 1973. Development of scion cultivars of citrus in Florida. *Proc. Fla. State Hort. Soc.* 86:84-88.
9. Horowitz, R. M. 1961. Studies on the structures and bitterness of the flavonoid glycosides of citrus. In G. Johnson and T. A. Geissman, "Symposium of biochemistry of plant phenolic substances." Colorado State Univ., Ft. Collins, Colorado. pp. 1-8.
10. Kefford, J. F. 1959. The chemical constituents of citrus fruits. *Adv. Food Research* 9:285-372.
11. Nagy, S. and H. E. Nordby. 1972. Saturated and monounsaturated long-chain hydrocarbon profiles of lipids from orange, grapefruit, mandarin and lemon juice sacs. *Lipids* 7:666-670.
12. Nishiura, M., J. Matsushima and N. Okudal. 1957. Studies on the identification of citrus species and the discrimination of hybrids from nucellar seedlings. 1. Color reaction method with leaf extracts. Bul. Hort. Div. Tokai-Kinki Agr. Expt. Sta. 4:19-26.
13. Pieringer, A. P. and G. J. Edwards. 1965. Identification of nucellar and zygotic citrus seedlings by infrared spectroscopy. *Proc. Amer. Soc. Hort. Sci.* 86:226-234.
14. Pieringer, A. P., G. J. Edwards, and R. W. Wolford. 1964. The identification of citrus species and varieties by instrumental analysis of citrus leaf oils. *Proc. Amer. Soc. Hort. Sci.* 84:204-212.
15. Stanley, W. L. and L. Jurd. 1971. Citrus coumarins. *J. Agr. Food Chem.* 19:1106-1110.
16. Tatum, J. H. and R. E. Berry. 1972. Six new flavonoids from citrus. *Phytochem.* 11:2283-2288.

PERFORMANCE OF 'PINEAPPLE' ORANGE AT THREE TREE SPACINGS[1]

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Abstract. **'Pineapple' orange trees on rough lemon rootstock were planted at spacings of 25′ X 20′, 20′ X 15′, and 15′ X 10′ in 1960. Earlier economic returns were realized from the closely planted trees since fruit yields per acre have been considerably higher. Yields obtained from the 2 closest spacings were similar in 1974. Fruit from the closest spacing has been larger with lower solids and later maturity. Hedging was started in 1966 in the closeest spacing and in 1971 in the intermediate spacing but it has not yet been necessary in the widest spacing.**

Increased costs of land, production and harvesting have led Florida citrus growers to seek the most effective use of land and the most efficient means of fruit production and harvesting. There is increased interest in close tree spacing as a means of accomplishing these goals (2). Higher initial yields are obtained by increasing the number of trees per acre, hence it is conceivable that growers could recover high investment and development costs in a shorter time. This early yield advantage tends to diminish, however, as the trees compete for light, water, and nutrients and may be reversed if the trees are allowed to become overcrowded (1). Development and use of dwarfing rootstocks is perhaps the key to success in closely spaced plantings (3).

A tree spacing experiment was initiated in 1960 and results published in 1969 indicated a strong correlation between tree number and fruit per acre (4). Now, 5 years later, it seems appropriate to reevaluate the spacings in the light of current data.

Materials and Methods

'Pineapple' orange trees on rough lemon rootstock were planted at spacings of 25′ X 20′, 20′ X 15′ and 15′ X 10′ at the Agricultural Research and Education Center grove near Davenport in 1960. Each plot consists of 2 rows of about 650 feet, bordered by buffer rows of the same spacing. The treatments are replicated 3 times in an area of approximately 10 acres. Measurements of fruit yield, fruit size, internal fruit quality, tree height and trunk circumference have been recorded and analyzed statistically. Each row received the same amounts of fertilizer and spray material so that

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