

EVALUATION OF 13 ROOTSTOCKS FOR 3 SWEET ORANGE CLONES AND A  
TANGOR CULTIVAR FOR TOLERANCE TO TRISTEZA VIRUS  
AT MALAMA-KI, HAWAII

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## ABSTRACT

Fifteen-year old 'Washington Navel', 'Valencia', 'Pera', and 'Ortanique' cultivars grafted on thirteen citrus rootstock species and hybrids were evaluated for tristeza tolerance at the University of Hawaii, Malama-Ki Experimental Farm. One hundred percent natural infestation of all experimental trees was detected and confirmed by use of Immunodiffusion test and the viral inclusion staining technique. Cultivars grafted on sour orange, 'Sampson' tangelo and Citrus amblycarpa rootstocks were lost due to tristeza prior to 1972. 'Batangas' and rough lemon were rootstocks that produced the most vigorous growth. 1978 seasonal production was high with trees on rough lemon, 'Batangas', 'Rangpur', 'Kona orange', 'Cleopatra' and Citrus sunki. Upright growth habit was observed with trees on 'Cleopatra' and 'Rangpur' rootstocks. The best quality fruit was harvested from trees grafted on Citrus sunki and 'Cleopatra' rootstocks. 'Heen naran' performed well with 'Washington Navel' but was not a satisfactory rootstock for the other three cultivars. In this experiment, Citrus taiwanica, 'Siameño' and 'Troyer' were not desirable rootstocks due to poor production. Citrus amblycarpa, 'Sampson' tangelo and sour orange were not suitable rootstocks for oranges and mandarins because of their susceptibility to tristeza virus.

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## I. INTRODUCTION

The history of Hawaiian citrus culture began in 1792, when seedlings of sweet orange, C. sinensis, L., were introduced by Captain George Vancouver from Tahiti. These plants were distributed throughout the Hawaiian Islands and were found to grow especially well in the Kona district of Hawaii (Crawford, 1937; Higgins, 1905).

During the period 1840 to 1870, oranges became the most important export item from Kona to California. The shift of interest to other more remunerative crops began after 1870 and the rapid development of a citrus industry in California led to decline in Hawaiian citrus culture. The discovery of the mediterranean fruit fly, Ceratitidis capitata, Wied., in 1910 resulted in a federal quarantine 2 years later and led to the end of citrus exports from Hawaii to the mainland United States (Pope, 1934; Flower & Jefferson, 1962).

Citrus growing in Hawaii is presently more of a back-yard crop than a commercial enterprise. In 1977, about 455,000 pounds of oranges and tangerines were produced in the state. In 1977, orange consumption in Hawaii was 14,272,000 pounds, of which more than 95% were imported (Hawaii Agricultural Reporting Service, 1978).

Oranges produced in Hawaii have a severe handicap in marketing because consumer preference is mainly guided by external appearance. Smooth, attractive, bright orange fruits are preferred over the large greenish yellow orange produced in Hawaii.

Poorly colored Hawaiian citrus are the result of high night temperatures of 13°C or above (Reuther & Rios., 1969). High relative humidity adds to this problem causing thin rind susceptible to blemishing.

In warm climates, there is a rapid decrease in total acids and granulation of pulp is common (Hayes, 1953; Reuther & Rios, 1969).

The use of a properly selected rootstock is important for specific locations, cultivars and disease resistance, especially virus diseases. Decline of sweet orange on sour orange rootstock is caused by tristeza virus, which limits the use of sour orange and other susceptible rootstocks, such as grapefruit, lemon and tangelo. The most recent outbreak of tristeza disease was recorded in Central Florida in 1973. Garnsey and Jackson (1976) estimated a total tree loss of 25,900 plants within a three year period which accounted for 31% of the citrus population in ten groves. 'Quick decline' symptom was common, with many trees deteriorating and dying within several months.

Ever since the infectious nature of the tristeza virus was demonstrated in 1946, studies have been conducted to find a solution to the problem. Use of virus tolerant rootstocks and cross protection with mild strains of tristeza have been practiced.

The purpose of this experiment was to compare the tolerance of thirteen citrus rootstocks to tristeza virus and the response of four cultivars on these rootstocks.

## II. LITERATURE REVIEW

Virus and virus-like diseases have caused greater losses in citrus than in any other tree crops. Tristeza virus is a destructive pathogen of citrus second only in economic importance to the Asian Greening disease in California (Roistacher, 1976). During the past 35 years, tristeza virus has destroyed more than 30 million trees in South America, South Africa, Spain, and the United States (Bitters, 1973).

The tristeza virus is approximately 2000 nm x 10 to 12 nm in size and resembles the beet yellow virus. It is usually found in the phloem tissues and the viral inclusion is placed in the 'Clostervirus' group (Bar-Joseph et. al., 1972; Christie & Edwardson, 1977). Tristeza virus is indigenous to Asia and was believed to be present in Australia as early as 1870. The citrus industry was not aware of the disease, probably because a majority of the citrus groves were established from marcotts and seedling plants which are not susceptible to tristeza infections (Webber, 1943).

The occurrence of gummosis disease caused by Phytophthora citrophthora Leon. and Phytophthora parasitica Dast. in the Mediterranean during 1834 to 1918, led to the use of sour orange, C. aurantium L., as a resistant rootstock (Batchelor & Rounds, 1948).

Tristeza decline of sweet orange grafted on sour orange rootstock was first recorded in South Africa in 1910. The virus was introduced to South America before 1930 (Wutscher, 1977) and in a twelve year period, more than 6 million trees were destroyed in the State of Sao Paulo, Brazil. This comprised about 75% of the citrus industry in that state (Bennett & Costa, 1949). The disease was given the name 'Tristeza',



which means sadness or melancholy in Spanish and Portuguese (Webber, 1943).

In California, the decline of trees on sour orange was observed in 1939 in Covina-Azusa and was named 'Quick Decline' (Bitters & Parker, 1953). Full details of the symptoms of tristeza infection have been described by Bennett and Costa (1949) and Salibe (1973).

The die-back of citrus caused by tristeza virus was initially considered as an incompatibility response between scion and stock. Toxopeus in 1937 (Bennett & Costa, 1949) suggested that a toxin produced by the sweet orange foliage was lethal to the sour orange rootstock. Other hypotheses included nutrient disorder, extreme pH and adverse soil conditions (Webber, 1943). The infectious nature of the decline was first suggested by Bitancourt and Fawcett (Bennett & Costa, 1949) in 1930. They believed the possible cause of the decline was due to a latent virus present in the sour orange. Webber in 1943 hypothesized that the virus was actually latent in the sweet orange and was able to cause sensitive responses in combination with sour orange. He further suggested that a possible viral inhibiting mechanism is present in the sour orange foliage which protects sour orange from tristeza infection.

In 1946, Fawcett and Wallace in the United States successfully demonstrated the infectious nature of tristeza disease by aphid transmission. The most effective vector was identified as Aphis citricidus Kirk., the 'Black Citrus aphid' (Fawcett & Wallace, 1946; Costa & Grant, 1951). Other aphid carriers include A. gossypii Glv., A. craccivora, A. spiraeicola Patch., and Toxoptera aurantii (Racah et. al., 1976; Knorr, 1977). Efficiency of virus transmission by A. citricidus Kirk. and A. gossypii Glv. was discussed by Bennett and Costa (1949), Costa

and Grant (1951), Dickson et. al. (1951) and Raccah et. al. (1976).

Bennett and Costa in 1947 (Bennett & Costa, 1949) confirmed the infectious nature of tristeza using both aphid transmission and tissue inoculation. The reports on different strains of tristeza were initiated by Grant and Costa in 1950 (Grant & Costa, 1951). Variation in severity of symptoms were observed when a virus infected 'Barao' orange was graft-inoculated onto non-tolerant 'Watt' tangelo. Further, transmission to plant from mildly infected trees produced only mild symptoms. Costa et al. in 1950 reported a possible relation between the stem pitting disease of grapefruit and tristeza. Fraser of Australia (McClellan & Van der Plank, 1955) reported on quantitative differences in tristeza symptoms in 1952. A tristeza strain which caused tristeza decline of sweet on sour orange also caused vein corking, stem splitting, stunted growth and yellowing of sour orange seedlings. The strain was named 'Seedling Yellows'.

McClellan and Van der Plank in 1955 postulated that the tristeza disease is caused by a virus complex composed of a seedling yellows component and a stem pitting component. They suggested that the stem pitting component could exist alone, causing stem pitting disease in all citrus species. Seedling yellows component, on the other hand, had not been observed without the stem pitting disease. It was also suggested to be the cause of tristeza decline. Evidence for this virus complex and the response of different citrus species to the complex was described by McClellan in 1977 (a, b) and by Salibe in 1973.

Anatomical symptoms of tristeza-infected 'Valencia' orange on sour orange rootstock were studied by Schneider in 1954 (a). He observed

severe sieve-tube necrosis immediately below the graft union extending 8 to 10 inches in the direction of the root. Schneider suggested that the decline was due to root starvation caused by phloem degeneration. Similar decline symptoms could also be caused by prolonged girdling of the trunk (Schneider, 1954 b).

Tristeza virus particles were first observed as flexuous rods in the phloem cells of 'Mexican' lime, *C. aurantifolia* L. (Kitajima et. al., 1965; Price, 1966). Bar-Joseph et. al. in 1972 purified and characterized these particles as RNA and a single species of protein. Garnsey et. al. (1977) were able to inoculate Citron, *C. medica* L., with these purified particles, and induce tristeza decline in 20 out of 67 attempts. This provided the first biological proof that the rod shaped particles were in fact the tristeza virus.

More rapid efficient assay procedures are now available to replace 'Mexican' lime as a tristeza indicator. Sodium Dodecyl Sulfate (SDS) Immunodiffusion test with tristeza anti-serum (Gonsalves et. al., 1978) and the tristeza viral inclusions staining with Azure A test (Garnsey, personal contact, 1979; Christie & Edwardson, 1977) have reduced the time required for identification of tristeza from 3 to 18 weeks for the 'Key' lime test (Wallace, 1968), to 1 to 2 days.

Since no control of tristeza decline was known and the presence of effective vector made it impractical to use virus free bud wood, rootstock studies conducted in many countries during the past 2 decades have concentrated on finding tristeza tolerant rootstocks.

A major difficulty in rootstock evaluation studies is in judging the horticultural characteristics of specific scion/rootstock combina-

tions. The screening of disease resistance to fungus and virus could be accomplished within a 3 to 4 year period by using young plants (Wutscher, 1979). To obtain any significant evaluation of specific scion-stock combinations requires however at least 10 to 15 years. Usefulness of the information acquired from these studies is limited to areas with relatively similar soils and environments.

The graftage of scion and stock represents an artificial symbiosis between the two individuals which is of mutual benefit. The graft union is completed by the growth of new cells with cell walls in close contact. The influence of scion and rootstock on each other is not completely understood, but is considered about as important as environmental effects (Joiner, 1955). Interactions of scion and rootstock are reciprocal. Characters expressed by one can be modified by the other (Hodgson and Cameron, 1943; Webber, 1948). Hodgson and Cameron (1943) demonstrated the influence of trifoliolate and rough lemon on tree size by reciprocal grafts of the two species. The degree of influence on tree size is largely dependent on the vigor of the scion cultivar. If a less vigorous scion is used, it will be the dominant factor and result in a dwarfed tree. Characters reported to be influenced by scion and stock effects include tree size (Hodgson & Cameron, 1943), root characters (Castle & Krezdorn, 1975), mineral composition in leaves (Smith, 1975), and holding capacity of fruit on tree (Hodgson & Egger, 1938; Hodgson, 1967; Webber, 1948).

Considerable studies on potential rootstocks have been conducted by various investigators in different areas. Some pertinent results of their investigations are reviewed here.

### Sour orange

Sour orange, C. aurantium L., in addition to being resistant to Phytophthora parasitica and other foot rot causing fungi (Hutchson & Grimm, 1973), is also resistant to Type A psorosis (Wutscher, 1979). Seeds of sour orange are numerous, usually around 40 per fruit. They are polyembryonic, producing 76 to 80% of nucellar embryos. Seeds germinate readily and can survive more unfavorable conditions than most citrus types (Webber, 1948). Sour orange as a rootstock is highly compatible with sweet orange, mandarin and grapefruit cultivars. Trees grafted on sour orange generally remain productive over a long period of time. Citrus fruits grown on this rootstock are medium in size with good quality (Gardner & Horanic, 1966). Except for lemon cultivars, all citrus on sour orange rootstock are highly susceptible to tristeza decline (Salibe, 1973). 'Eureka' lemon on this rootstock is susceptible to exocortis virus (Bitters & Batchelor, 1951). Sour orange is also susceptible to the citrus and burrowing nematodes.

### Citrus taiwanica

Citrus taiwanica, Hort. ex. Tanaka, is a natural hybrid of sour orange native to Taiwan. It adapts readily in sub-tropical climates and grows to a large size. C. taiwanica is relatively tolerant to tristeza, but sensitive to rootrot and nematode infection (Wutscher, 1979). Fruits produced on this rootstock are usually low in total acids and soluble solids, especially with 'Valencia' orange and grapefruit (Wutscher & Shull, 1973; Wutscher & Dube, 1977; Wutscher et. al., 1975).

### Rough lemon

Rough lemon, C. limon, L., is native to north-eastern India. It is widely used as rootstock for citrus in Argentina, Australia, Brazil, Florida and India. Seeds of rough lemon are small, highly polyembryonic and 90 to 100% nucellar (Webber, 1932; Hodgson, 1967). Seedlings are vigorous, with an upright trunk. They are graft-compatible with most citrus cultivars. Rough lemon as stock usually produces vigorous vegetative growth and large fruit size (Bitters & Batchelor, 1951; Weir, 1976). Fruits from this stock are usually somewhat low in total soluble solids and acids and have a relatively short holding period on the tree after maturity. Rapid drying and granulation of pulp often occurs soon after maturity (Bartholomew et. al., 1948; Johnson, 1953; Economides, 1976). Except for grapefruit, all citrus on rough lemon stock are tolerant to tristeza decline and xyloporosis virus (Joiner, 1955). Rough lemon hybrid selections 'Milam' and 'Estes' were reported to be resistant to burrowing nematode (Hodgson, 1967). Production life of citrus on rough lemon rootstock was relatively short, peak production occurring between the tenth and twenty-fifth years and declining thereafter. Trees on this rootstock were susceptible to gummosis (Pope, 1934) and young tree decline (Lawrence & Bridge, 1973). Lemon cultivars on rough lemon rootstock are susceptible to Type A psorosis (Joiner, 1955).

### Rangpur

'Rangpur', C. reticulata var. austera, Swingle, is a seedy mandarin or mandarin hybrid native to India. It is the major rootstock for citrus

in Brazil (Moreira & Salibe, 1969) and is used to some extent in S.E. Asia (Cohen, 1970). 'Rangpur' can adapt to a wide range of soil type and is tolerant to tristeza virus and phytophthora root rot. Sweet oranges and mandarins, especially the mid-season cultivars, grow well on this stock. 'Rangpur' stock stimulates early fruiting and results in high quality fruits (Webber, 1948; Lawrence & Bridge, 1973).

Virus free bud wood is highly recommended for 'Rangpur' because of its susceptibility to exocortis and xyloporosis virus (Moreira and Salibe, 1969; Salibe, 1973).

#### Sweet orange

Sweet orange, C. sinensis, L., is native to southern China. It was the most popular rootstock in California in the early 1900's (Webber, 1948). Sweet orange grows best in rich sandy soil or heavy soil with good drainage. Trees on this stock are usually large, vigorous and long-lived. Fruits are medium in size with excellent flavor and quality. Holding period of fruits on trees of sweet orange rootstock is long, with less problems of drying and granulation than with other rootstocks. Except for sensitivity to phytophthora root rot, sweet orange is a very satisfactory rootstock for sweet orange cultivars (Bitters & Batchelor, 1951).

Sweet orange is tolerant to tristeza, exocortis and xyloporosis virus and resistant to young tree decline and sour orange scab (Wutscher, 1979).

## Mandarin

Mandarins in general are satisfactory stocks for most citrus types (Grant et. al., 1949). Mandarin seeds are highly polyembryonic, with 80 to 100% of nucellar seedlings. Trees on mandarin stock are tolerant to gummosis, citrus scab, and tristeza virus. Mandarin rootstocks usually produce small compact trees (Webber, 1923, 1948). The slow growing habit of mandarin rootstocks which may be a limiting factor under strictly sub-tropical condition does not appear to be a problem in Hawaii.

## Cleopatra mandarin

'Cleopatra' mandarin, C. reticulata, Blanco, is native to India. It was introduced to Florida in 1888 and was considered a promising replacement rootstock for sour orange (Webber, 1948; Burke, 1967).

Compared to trees on sweet orange, 'Cleopatra' requires a longer growing period to achieve full production. Yields on this rootstock are usually low during the first 5 years, but gradually increase as the tree matures (Hodgson, 1967). Fruit quality on this rootstock is excellent with high total soluble solids and acids. 'Cleopatra' is a suitable rootstock for sweet orange, mandarin and lemon cultivars (Economides, 1976), but not for grapefruit and 'Valencia' orange because of low yields (Wutscher & Shull, 1970).

'Cleopatra' mandarin is tolerant to tristeza and xyloporosis with low incidence of blight - young tree decline (Johnson, 1953; Lawrance & Bridge, 1973; Bitters & Batchelor, 1951). It is also tolerant to phytophthora, but susceptible to the citrus and burrowing nematodes



(Wutscher, 1979; Baines et al., 1957).

### Citrus sunki

Citrus sunki, Hort. ex. Tanaka., is a mandarin stock used extensively for 'Ponkan' mandarin in China (Bitters, 1974). It was proven to be feasible as a rootstock for grapefruit (Wutscher & Shull, 1975; Gardner and Horanic, 1966), as well as for sweet oranges and mandarins (Salibe, 1973; Wutscher, 1979).

Citrus sunki is tolerant to exocortis and tristeza virus, but susceptible to phytophthora root rot (Wutscher & Shull, 1975). Trees on this rootstock have been reported to have a short productive period (Webber, 1948).

### Batangas mandarin

'Batangas' mandarin, C. reticulata, Blanco, is a vigorous upright tree four meters tall or higher, which originated in the Philippines. 'Batangas' is resistant to root diseases, tolerant to tristeza and high temperature, but sensitive to drought. It also has a tendency to alternate bearing (Hodgson, 1967). 'Batangas' is not a suitable rootstock for 'Valencia' orange due to low production and susceptibility to exocortis virus (Wutscher & Shull, 1970).

### Sampson tangelo

'Sampson' tangelo, C. paradisi 'Sampson' x C. reticulata 'Dancy' is a low spreading tree with seedy fruits. Seeds have 100% nucellar embryos, but its value as rootstock is limited.

When 'Sampson' tangelo is used as a rootstock for sweet orange, it is similar to sour orange in susceptibility to tristeza virus (Webber, 1943). When 'Sampson' tangelo is used as a scion to sour orange rootstock, the combination is highly resistant to the disease (Bennett & Costa, 1949).

'Sampson' tangelo is a suitable stock for lemon cultivars. Fruits produced on this rootstock have high juice content with good quality and are resistant to shell bark and lemon decline (Bitters and Batchelor, 1951; Johnson, 1953).

### Citranges

Citranges are F<sub>1</sub>-hybrids of C. sinensis and Poncirus trifoliata, L., Osbeck. They resemble trifoliolate orange in leaf morphology, but stay evergreen like sweet orange (Swingle, 1967). Citranges are highly apomictic, with 100% nucellar seedlings (Tanaka, 1948; Webber, 1932). Citranges are compatible with most citrus cultivars with the exception of 'Eureka' lemon (Bitters & Batchelor, 1957; Baines et. al., 1957), and Chironja (R. A. Hamilton, personal communication, 1979). 'Troyer' and 'Carizzo' are the best known citrange cultivars. They are selections from the same cross of 'Washington Navel' orange with Poncirus trifoliata (Olson, 1960).

Trees on citrange rootstock are sometimes dwarfed. They produce better quality 'Valencia' oranges and grapefruit than 'Cleopatra' (Wutscher & Shull, 1972; Wutscher et. al., 1975, Phillip & Castle, 1977). As a rootstock, citranges were reported to out-yield sour orange (Webber, 1948; Gardner & Horanic, 1967; Colburn et. al., 1970).

Citranges are considered somewhat tolerant but not immune to tristeza virus. 'Troyer' is more tolerant to shell bark and exocortis virus, intermediate in tolerance to foot rot, but susceptible to the citrus and burrowing nematodes (Wutscher, 1979). 'Carizzo' citrange is more resistant to nematodes and tristeza. 'Morton' citrange is highly susceptible to gummosis (Baines et. al., 1957).

### III. MATERIALS AND METHODS

The rootstock study was initiated in 1961 at the Malama-Ki Experimental Farm on the Island of Hawaii. The project was assigned to the author in 1977 as a thesis problem for the degree of Master of Science in Horticulture.

Elevation of the Experimental Farm is three hundred feet above sea-level. Mean monthly temperature recorded in the past 10 years ranged from 18°C to 27°C. Average monthly relative humidity was approximately 82%. Annual rainfall was 275 cm (110 inches) and is most abundant from October to February. Soil type in the area belongs to the 'Malama extremely stony muck' soil (Soil survey of Island of Hawaii, 1973).

Thirteen citrus species and hybrids were included as rootstocks. These were:

- Sour orange, C. aurantium, L.,
- Citrus taiwanica, Hort. ex. Tanaka,
- 'Kona' sweet orange, C. sinensis, L.,
- Rough lemon, C. limon, L.,
- 'Cleopatra' mandarin, C. reticulata Blanco.,
- 'Rangpur', C. reticulata var. austera, Swingle,
- 'Heen naran' mandarin, C. lycopersicaeformis, Hort. ex. Tan.,
- 'Batangas' mandarin, C. reticulata, Blanco.,
- Citrus sunki, Hort. ex. Tanaka,
- Citrus amblycarpa, Hort. ex. Tanaka,
- 'Siamelo' (C. grandis x C. reticulata 'King Siam'),
- 'Sampson' tangelo (C. paradisi 'Sampson' x C. reticulata 'Dancy' Swingle),

'Troyer' citrange (C. sinensis x Poncirus trifoliata,  
L.Osbeck).

All seedling stocks were transplanted between September of 1961 to March of 1962. Trees were planted in four rows from North to South. In May of 1963, all rootstocks were grafted with virus free nucellar strains of 'Washington "Frost" Navel' orange, 'Valencia', 'Pera' and 'Ortanique' tangor. The treatments were replicated twice, giving a total of one-hundred and four trees.

Grove care included three to four applications of complete fertilizer (13-13-13) (5 lb/tree) and three to four insecticide sprays per year.

#### Tree Growth and Fruit Analysis

The 1978 harvest of Navel orange was recorded in November, 1978. The other three cultivars were harvested in February, 1979. The number of fruit on each tree was counted and the total yield was calculated by multiplying the number of fruits by the average fruit weight.

Fruit quality analysis was based on samples of ten fruits harvested at random from each experimental tree. Each fruit was weighed and the juice extracted. Fifteen ml of juice from each of the ten fruits were then transferred into a beaker and homogenized. Total soluble solids of the homogenized juice was determined by use of a hand refractometer. Total acidity was determined by titrating fifteen ml of the homogenized juice with 0.1 N NaOH, using phenolphthalein as the indicator (Soule & Lawrence, 1959).

Brix/acid ratio was calculated by dividing the percent of total soluble solids by the percent of total acids.

Other morphological characters were measured in June, 1978. These included tree height, canopy diameter and trunk girth ratio. The canopy volume of each tree was computed from the measurement of tree height and canopy diameter using the formula:

$$\frac{(\text{Width of Canopy})^2 \times (\text{Height of Canopy})}{4} = \text{Volume of Canopy}$$

(Wutscher & Shull, 1970). Tree canopy volume was recorded in units of cubic meters.

The bearing density of the tree canopy of each scion/rootstock combination was obtained from dividing the total weight of fruit by the volume of the canopy.

Trunk girth ratio was computed as the ratio between the circumference of the scion 10 cm above the graft union and the circumference of the rootstock 10 cm below the graft union.

All data collected were analyzed as a split plot design using the Statistical Analysis System (SAS) computer package, with scion cultivars as the main plot and rootstock as the sub-plot.

### Tristeza Virus Assays

Young shoots and fruits were collected from each treatment tree for tristeza virus assays during Fall of 1978 and Spring of 1979. The two assaying techniques were used to provide rapid identification of tristeza virus infections and no quantitative values were assigned.

Immunodiffusion test with sodium dodecyl sulfate (SDS) treated virus extract was the first method (Purcifull & Batchelor, 1977). The tristeza antiserum used was prepared from Florida T-4 strain tristeza and was obtained along with extracts from healthy citrus and tristeza carrying citrus through Dr. D. Gonsalves of the New York State Agricultural Experiment Station. Preparation of the antiserum was described by Purcifull and Batchelor (1977) and Gonsalves et. al. (1978).

Preparation of Immunodiffusion gel: To prepare about 40 plates (Plastic petri plates, 100 x 15 mm), 4 grams of Noble agar (Difco) was added to 300 ml of deionized water and autoclaved for five minutes at 121°C (250°F). After autoclaving, 5 g of sodium azide (Mallinckrodt, Inc.,) was added and stirred till dissolved. To another 150 ml of hot deionized water, 2.5 g of sodium dodecyl sulfate (Mallinckrodt, Inc.) was added and stirred till dissolved, then added to the agar-azide mixture and brought up to a final volume of 500 ml with hot deionized water. The medium was then placed in petri plates at about 12 ml per plate.

After the agar solidified, reactant wells were cut in the agar with a No. 4 cork borers, and the agar plugs were aspirated with a glass tubing connected to a vacuum line. The gel pattern consisted of a central anti-serum well surrounded by 6 peripheral antigen wells (each well is 7 mm in diameter) with the peripheral wells 5 mm away from the central well at the closest point. Antiserum was loaded into the central well and antigens into each of the peripheral wells. Two of the peripheral wells were reserved for controls, one with extract from healthy citrus trees and a second with extract from trees known to carry tristeza virus.

Preparation of antigen: For each plant sample, one gram of soft bark tissue from new growth was ground in 2 ml of 0.2% sodium dodecyl sulfate solution with a mortar and pestle. Plant extract was screened through cheesecloth and loaded into reactant wells by means of disposable pipets. If the tissue contained tristeza virus, immunoprecipitation was visible within 12 to 24 hours at room temperature of approximately 24°C (Purcifull & Batchelor, 1977; Gonsalves et.al., 1978).

The second tristeza virus assay used in this experiment was by inclusion staining with Azure A. A total of 30 tree samples taken at random were tested. Free-hand sections of leaf petiole and young fruits were placed into a stain solution composed of 5 drops of Azure A stain (0.1 g in 100 ml 2-methoxyethanol) and 1 drop of 0.2 M  $\text{Na}_2\text{PO}_4$  for thirty minutes. The stained tissues were then transferred into 95% ethanol for fifteen minutes and then rinsed 2 to 10 seconds in 2 methoxy-ethyl-acetate. The treated tissues were mounted in Euparal and scanned under 40 to 100 magnification of a compound microscope for stained purple streaks or clusters of tristeza viral inclusions (Garnsey, personal communication, 1979; Christie & Edwardson, 1977).



#### IV. RESULTS AND DISCUSSION

Seventy-nine trees of the original 104 planted were still alive and growing in the field in February, 1979. No gummosis disease symptoms were detected on any of the rootstocks, probably because of the porosity of the 'Malama extremely stony muck' soil (Soil Survey of Island of Hawaii, 1973) in the experimental field. This soil type is exceptionally well-drained with practically no accumulation of free water around tree trunks. This minimized the danger of phytophthora infection. On this highly leached soil, symptoms of nutrient deficiency develop readily. Because of this, supplemental applications of nitrogen and trace minerals were necessary after periods of heavy rainfall.

One tree of 'Pera' on rough lemon rootstock died in 1965 and was not replaced. All trees on sour orange, 'Sampson' tangelo and C. amblycarpa were discarded prior to the termination of the experiment. Symptoms of stem pitting and stunted growth were observed by Dr. R. A. Hamilton in 1972. This decline was presumably caused by tristeza infection.

The presence of tristeza virus in the entire experimental population was verified in the Fall of 1978 by use of the Immunodiffusion test with sodium dodecyl sulfate treated plant extracts. Plant extract treated with 0.2% sodium dodecyl sulfate reacted positively to tristeza antiserum and immunoprecipitations were visible in all samples tested.

A second test using Azure-A-stained leaf petioles and fruit sections revealed purple streaks of viral inclusions in all 30-sample trees tested, confirming results of the Immunodiffusion test.

Nucellar and virus indexed bud-wood was presumably free from tristeza when the field planting was made. Tristeza infection was from natural inoculation and occurred at random during the period from 1961 to 1978. Because of differences in age of onset of tristeza virus, production of each scion and rootstock combination in the early years were not used. Trees infected as young trees might have been more severely affected by tristeza than those infected at a later date. However, based on the observations of probable tristeza decline on sour orange, 'Sampson' and C. amblycarpa rootstocks in 1972, tristeza was probably widespread in the experimental orchard prior to 1972. Dickson et. al. (1951) reported that each tristeza infected tree has a potential of infecting 2 trees within one year. This value was based on the low incidence of transmission by A. gossypii Glov. in Southern California (Roistacher, 1976). Since a more effective vector, A. citricidus, is present in Hawaii (Bennett & Costa, 1949), the entire experimental population was probably infected between 1968 to 1974.

Parameters of tree growth and production measured in 1978 can therefore be considered as valid observations on preliminary evaluation of tristeza tolerance of each scion/rootstock combination.

In this experiment, trees of 'Washington Navel' and 'Pera' had taller trees and larger canopies than those of 'Valencia' and 'Ortanique'. This could possibly be due to the influence of less vigorous scion of 'Valencia' and 'Ortanique'. Rootstocks which enhanced vegetative growth included rough lemon, 'Batangas', 'Rangpur', and 'Cleopatra'. Rough lemon was the most vigorous stock. No significant differences in volume of tree canopy were found between rootstocks of 'Kona orange', 'Siameo',

'Heen naran' and Citrus sunki. Citrus taiwanica and 'Troyer' rootstocks produced relatively small canopies (see Table 1).

The trunk girth ratio was determined by dividing the trunk girth above the graft union by the trunk girth below. Overgrown scion have ratios greater than one and over grown rootstocks have ratios less than one.

In this experiment, higher trunk girth ratio was most frequent with trees of 'Pera' orange. In trees of 'Ortanique', the rootstock outgrew the scion. Rootstocks which encouraged greater scion than stock growth included C. sunki, 'Rangpur' and 'Kona' orange. The largest stock girth was found on 'Troyer' (see Table 1), which had the lowest girth ratio among various rootstocks. Mendel (1969) suggested in 1969 that overgrowth of the scion or rootstock portion of a grafted citrus is largely a morphological characteristic. Exocortis virus, tristeza virus, girdling of tree trunk and improper budding height are all possible causes for overgrowth of the stock by the scion (Knorr, 1973; Schneider, 1954 a, b; Labanauskas et. al., 1976). Stock overgrowth occurs principally when slow growing rootstocks, such as citrange and Poncirus trifoliata are grafted to vigorous scion cultivars. Increase in photosynthetic products from the scion stimulates growth of the rootstock (Gardner & Horanic, 1967).

Rootstocks appeared to influence productivity more than the scion cultivar used. All four cultivars in this experiment produced significantly more fruit on rough lemon rootstock. Next in order were 'Batangas', 'Rangpur', 'Kona orange', 'Cleopatra', and C. sunki. Trees on 'Heen naran' and 'Troyer' rootstocks were intermediate in

Table 1. --Average tree height, canopy volume and trunk girth ratio of scion cultivars and rootstocks.

Rootstocks	Tree Height (meters) C.V. = 12.37	Canopy Volume (cubic meters) C.V. = 28.06	Trunk Girth ratio C.V. = 7.11
Rough lemon	3.4a <sup>y</sup>	14.4a <sup>y</sup>	0.99 bc <sup>y</sup>
Rangpur	3.3ab	10.9 b	1.07ab
Kona orange	2.9 bc	8.0 c	1.05abc
<u>Citrus taiwanica</u>	2.4 d	4.3 de	0.88 d
Siameño	2.6 cd	7.3 cd	0.99 bc
Troyer citrange	2.3 d	4.9 de	0.82 d
Batangas mandarin	3.2ab	13.6ab	0.98 c
<u>Citrus sunki</u>	2.4 d	6.4 cd	1.12a
Cleopatra mandarin	3.3ab	11.4 b	0.99 bc
Heen naran mandarin	2.6 cd	6.1 cde	0.99 bc
<b>Scion Cultivars</b>			
Washington Navel	3.1a <sup>y</sup>	11.1a <sup>y</sup>	0.98 b <sup>y</sup>
Valencia orange	2.5 b	5.5 b	0.98 b
Pera orange	3.1a	10.6a	1.10a
Ortanique	2.7 b	7.5ab	0.90 c

<sup>y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

productivity. The lowest production was on C. taiwanica and 'Siamelo' rootstocks.

No significant differences in bearing density were observed between different rootstocks. This may be due to insufficient replication and the high degree of variation noted (C.V. = 48.0) (see Table 2).

Data on fruit quality are presented in Table 3. Fruits of Navel orange were larger than those of 'Valencia', 'Ortanique', and 'Pera'. Rootstocks which influenced the production of large fruits included 'Batangas', C. sunki, rough lemon, 'Troyer', 'Siamelo', and 'Heen naran'. Scion cultivars grafted on 'Kona orange' produced the smallest fruits among the 10 rootstocks observed (see Table 2).

Rootstocks which influenced the production of fruits with high total soluble solids were C. sunki, 'Cleopatra', 'Heen naran', 'Kona orange', and 'Troyer'. Fruits produced on rough lemon, 'Batangas', 'Siamelo', and 'Rangpur' rootstocks had somewhat lower total soluble solids (see Table 3).

Among the four scion cultivars, fruits of 'Pera' and 'Ortanique' had higher total soluble solids than those of 'Washington Navel' and 'Valencia' (see Table 3).

'Valencia' orange had the highest total acid content among scion cultivars. An average of 1.63% of total acid was recorded in 1978. The brix/acid ratio of 6.4 to 1 suggest that fruits of 'Valencia' may have been harvested prematurely and the high acidity may have been due to immature fruit.

'Pera' sweet orange was also high in total acidity, with an average of 1.16%, followed by 'Ortanique' with 0.95%. 'Washington Navel'

Table 2. --Average yield per tree, bearing density of the canopy and average fruit weight of each of the scion cultivars and rootstocks for the 1978 harvest

Rootstocks	Yield (1978) (kg) C.V. = 40.74	Bearing density (kg/m <sup>3</sup> ) C.V. = 48.0	Average Fruit Weight (g) C.V. = 5.0
Rough lemon	50.56a <sup>Y</sup>	3.73 ns <sup>Z</sup>	230.6ab <sup>Y</sup>
Rangpur	39.07ab	3.77	222.3 bc
Kona orange	38.49ab	5.34	212.8 c
<u>C. taiwanica</u>	17.90 c	4.94	218.5 bc
Siameño	19.32 c	3.18	232.8ab
Troyer	24.60 bc	5.80	229.0ab
Batangas	48.39a	3.68	237.9a
<u>C. sunki</u>	37.60ab	6.04	232.5ab
Cleopatra	38.10ab	3.65	218.3ab
Heen naran	25.52 bc	5.86 ns	228.8ab
<hr/>			
Scion Cultivars			
Washington Navel	34.40ab <sup>Y</sup>	3.58 c <sup>Y</sup>	290.0a <sup>Y</sup>
Valencia orange	22.60 b	4.45 b	205.0 b
Pera orange	33.80ab	3.72 c	187.0 b
Ortanique	46.60a	7.25a	207.0 b

<sup>Y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>Z</sup>ns: no significant difference at 5% level.

Table 3. --Average total soluble solids, total acidity and brix/acid ratio of scion cultivars and rootstocks in 1978

Rootstocks	Total Soluble Solids (%) C.V. = 3.18	Total Acids (%) C.V. = 11.06	Brix/Acid ratio C.V. = 8.66
Rough lemon	9.5 e <sup>y</sup>	1.03 b <sup>y</sup>	10.74 bc <sup>y</sup>
Rangpur	10.2 d	1.16ab	10.76 bc
Kona orange	10.6 bc	1.16ab	10.34 c
<u>C. taiwanica</u>	10.3 cd	1.03 b	11.04 bc
Siameño	10.1 d	1.13ab	10.58 bc
Troyer	10.6 bc	1.07ab	11.60 b
Batangas	10.2 d	1.16ab	10.10 c
<u>C. sunki</u>	11.0a	1.19a	10.98 bc
Cleopatra	10.9ab	1.08ab	11.78ab
Heen naran	10.8ab	1.19a	10.39 c
Scion Cultivars			
Washington Navel	10.2 b <sup>y</sup>	0.72 d <sup>y</sup>	14.80a <sup>y</sup>
Valencia orange	10.3 b	1.63a	6.40 d
Pera orange	10.8a	1.16 b	10.00 c
Ortanique	10.6a	0.95 c	12.30 b

<sup>y</sup>Mean separation in column by Duncan's multiple range test, 5% level.

was lowest, with 0.72%.

Variation in acid content was not significant between fruit produced on C. sunki, 'Heen naran', 'Batangas', 'Rangpur', 'Kona orange', 'Siamelo' and 'Cleopatra' rootstocks. Fruits produced on rough lemon and C. taiwanica were relatively low in acidity (see Table 3).

### Washington Navel

Under these experimental conditions, the best production of 'Washington Navel' was on rough lemon rootstock. The average yield of the 1978 harvest on this rootstock was 51 kg per tree (see Table 4).

Dwarfing effect of rootstocks was not observed with 'Washington Navel'. Trees on rough lemon, 'Rangpur', 'Cleopatra', 'Heen naran', and 'Batangas' rootstocks all exceeded three meters in height, with canopies exceeding twelve cubic meters. Trees on 'Rangpur' and 'Heen naran' were more upright in growth habit than on the other seven rootstocks. Medium sized Navel orange trees were produced on 'Siamelo', 'Kona orange', 'Troyer', C. taiwanica, and C. sunki (see Table 4).

No significant differences were observed in bearing density between rootstocks.

Smooth graft unions were observed with 'Washington Navel' on most rootstocks except 'Troyer', which had larger stock girth. This is contrary to the report of smooth graft union of Navel on 'Troyer' (Economides, 1976).

'Washington Navel' orange produced large fruit on 'Troyer', rough lemon, C. sunki, 'Batangas', and 'Cleopatra' rootstocks. Smaller fruits were produced on 'Kona orange'. No statistical difference in total



Table 4. --Main effects of rootstock on tree height, canopy volume, trunk girth ratio and yield of 'Washington "Frost" Navel' orange in 1978

Rootstocks	Tree Height (meters)	Canopy Volume (cubic meters)	Trunk Girth ratio	Yield (1978) (kg)
Rough lemon	3.7a <sup>y</sup>	17.95a <sup>y</sup>	1.01ab <sup>y</sup>	51.1 ns <sup>z</sup>
Rangpur	3.6a	12.95abc	1.11a	43.3
Kona orange	2.9 b	8.75 cde	1.01ab	38.2
<u>C. taiwanica</u>	2.9 b	7.00 e	1.03ab	32.1
Siameño	2.9 b	11.45 bcd	0.98ab	21.7
Troyer	2.6 b	7.35 de	0.81 c	25.1
Batangas	3.1a	14.20abc	0.96ab	37.5
<u>C. sunki</u>	2.8 b	6.30 e	1.08ab	36.9
Cleopatra	3.4a	16.75ab	1.03ab	34.3
Heen naran	3.4a	12.55abcd	0.92 b	33.4 ns

<sup>y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>z</sup>ns: no significant difference at 5% level.

soluble solids and total acids were found due to rootstocks used. The highest average total soluble solids reading of 11% was found in fruits produced on C. sunki rootstocks. Total acidity was highest in fruits produced on 'Troyer' and 'Batangas' rootstocks. Navel oranges harvested from rough lemon rootstocks were relatively low in both soluble solids and acids (see Table 5).

Brix/acid ratios were high in Navel orange regardless of rootstocks, with the highest brix/acid ratio detected in fruits produced on 'Cleopatra' rootstock, followed by C. sunki, and 'Siamelo' (see Table 5).

#### Valencia orange

'Valencia' produced the highest yields on 'Rangpur', 'Cleopatra' and rough lemon in 1978. Production on these rootstocks was however not statistically different from the others. Citrus taiwanica and 'Heen naran' appeared to be unsuitable rootstock for 'Valencia' orange because of poor yields.

'Valencia' orange trees on 'Rangpur' were the most vigorous in the experiment. Average height of this scion/stock combination was 3.23 meters, with a canopy of 11 cubic meters. It was followed by trees on rough lemon, and 'Cleopatra'. Trees on 'Siamelo', C. taiwanica, 'Troyer', 'Heen naran' and C. sunki were smaller and somewhat dwarfed.

Trunk girth ratio varied with different rootstocks. 'Valencia' on rough lemon, 'Rangpur', 'Kona orange', 'Siamelo', 'Batangas', and 'Heen naran' all resulted in relatively smooth graft unions. Scion overgrowth was observed with C. sunki and obvious overgrowth by the

Table 5. --Main effects of rootstock on bearing density of tree canopy, fruit weight, total soluble solids, total acids and brix/acid ratio of 'Washington "Frost" Navel' in 1978

Rootstocks	Bearing Dens. (kg/m <sup>3</sup> )	Fruit Wt. (grams)	T.S.S. (%)	T.A. (%)	Brix/Acid ratio
Rough lemon	2.97 ns <sup>Z</sup>	318.5a <sup>Y</sup>	9.18 ns <sup>Z</sup>	0.63 ns <sup>Z</sup>	13.9 d <sup>Y</sup>
Rangpur	3.63	263.5 de	9.78	0.72	13.2 d
Kona orange	4.47	255.3 e	10.56	0.71	13.8 d
<u>C. taiwanica</u>	4.61	284.2 bcde	10.34	0.74	15.0 bcd
<u>Siameño</u>	1.89	269.6 cde	10.38	0.71	16.4ab
Troyer	3.50	318.6a	10.23	0.81	13.6 d
Batangas	2.70	308.8ab	9.90	0.81	13.2 d
<u>C. sunki</u>	5.70	313.9ab	11.03	0.76	16.3abc
<u>Cleopatra</u>	2.04	296.8abc	10.22	0.64	17.4a
Heen naran	3.10	273.8 cde	10.03	0.69	14.3 cd

<sup>Y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>Z</sup>ns: no significant difference at 5% level.

rootstock was observed with 'Troyer' and C. taiwanica (see Table 6).

Fruit size was relatively uniform on all of the rootstocks except for 'Cleopatra', which produced the smallest fruits (see Table 7). Significant differences were not observed in total soluble solids regardless of rootstocks used. Lowest total soluble solids readings were from trees on rough lemon, and highest on trees from 'Cleopatra'. Relatively high acid content was observed from all fruits harvested in 1978. Fruits on trees from 'Rangpur' had the highest acid content (1.84%) and those on rough lemon were the lowest (1.45%) (see Table 7). All 'Valencia' fruits appeared to have been harvested prematurely, since brix/acid ratios were lower than the legal standard of 8 to 1 for naturally colored fruits (Soule and Lawrence, 1959).

#### Pera orange

Citrus sunki, rough lemon, 'Cleopatra', 'Batangas', 'Kona orange', and 'Rangpur' rootstocks produced good crops of 'Pera' orange in 1978. Among these rootstocks rough lemon and 'Batangas' induced the most vigorous growth with an average tree height of more than 3.3 meters and a canopy of more than 16 cubic meters. Tree size of 'Pera' orange on 'Rangpur', 'Cleopatra', 'Kona orange', and C. sunki was medium, with a canopy of approximately 10 cubic meters. Tree growth was more upright with 'Kona orange' and 'Cleopatra' (see Table 8). No significant differences in yield, bearing density, and total soluble solids were observed in 'Pera' on these rootstocks. Total acids was highest with fruits from trees on 'Kona orange' and lowest from 'Cleopatra' (see Table 9).

Table 6. --Main effects of rootstock on tree height, canopy volume, trunk girth ratio and yield of 'Valencia' orange in 1978

Rootstocks	Tree Height (meters)	Canopy Volume (cubic meters)	Trunk Girth ratio	Yield (1978) (kg)
Rough lemon	3.2ab <sup>y</sup>	9.60ab <sup>y</sup>	0.95 bcd <sup>y</sup>	31.1 ns <sup>z</sup>
Rangpur	3.2ab	11.00a	1.07ab	34.6
Kona orange	2.6abcd	4.00 c	1.07ab	19.3
<u>C. taiwanica</u>	2.3 cd	2.90 c	0.86 d	8.5
Siamelo	2.4 cd	4.45 bc	0.98 bcd	20.0
Troyer	2.1 d	3.85 c	0.85 d	21.7
Batangas	2.4 bcd	5.35 bc	1.02abc	23.8
<u>C. sunki</u>	2.0 d	4.55 bc	1.15a	26.3
Cleopatra	2.9abc	7.55 bc	0.90 cd	32.0
Heen naran	2.2 cd	3.20 c	0.97 bcd	14.4 ns

<sup>y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>z</sup>ns: no significant difference at 5% level.

Table 7. --Main effects of rootstock on bearing density of tree canopy, fruit weight, total soluble solids, total acids and brix/acid ratio of 'Valencia' orange in 1978

Rootstocks	Bearing Dens. (kg/m <sup>3</sup> )	Fruit Wt. (grams)	T.S.S. (%)	T.A. (%)	Brix/Acid ratio
Rough lemon	3.34 ns <sup>Z</sup>	204.8abc <sup>Y</sup>	9.52 ns <sup>Z</sup>	1.45 c <sup>Y</sup>	6.8 ns <sup>Z</sup>
Rangpur	3.18	216.5ab	10.10	1.84a	5.5
Kona orange	4.84	205.6abc	10.23	1.69ab	6.4
<u>C. taiwanica</u>	2.94	202.5abc	10.00	1.49 bc	6.7
Siameño	4.58	205.8abc	9.86	1.52abc	6.8
Troyer	6.03	202.6abc	10.09	1.58ab	7.4
Batangas	4.44	222.0a	10.10	1.64ab	6.1
<u>C. sunki</u>	5.71	214.5ab	10.58	1.64ab	6.6
Cleopatra	4.28	187.3 c	10.67	1.61ab	6.2
Heen naran	4.49	198.6abc	10.11	1.77ab	6.3

<sup>Y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>Z</sup>ns: no significant difference at 5% level.

Table 8. --Main effects of rootstock on tree height, canopy volume, trunk girth ratio and yield of 'Pera' orange in 1978

Rootstocks	Tree Height (meters)	Canopy Volume (cubic meters)	Trunk Girth ratio	Yield (1978) (kg)
Rough lemon	3.43ab <sup>y</sup>	18.65a <sup>y</sup>	1.08 bc <sup>y</sup>	43.7a <sup>y</sup>
Rangpur	2.90abc	10.80 b	1.28a	34.7ab
Kona orange	3.51a	11.55 b	1.20ab	34.3ab
<u>C. taiwanica</u>	2.64 bc	5.60 cd	0.94 cd	16.3ab
<u>Siameño</u>	2.19 c	4.50 cd	1.04 c	6.7 b
Troyer	2.49 c	5.50 cd	0.85 d	12.4ab
Batangas	3.35ab	16.85a	1.07 bc	40.2a
<u>C. sunki</u>	2.80abc	9.20 bc	1.23ab	44.9a
<u>Cleopatra</u>	3.51a	11.80 b	1.09 bc	42.4a
Heen naran	2.79abc	4.50 cd	1.21ab	19.5ab

<sup>y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

Table 9. --Main effects of rootstock on bearing density of tree canopy, fruit weight, total soluble solids, total acids and brix/acid ratio of 'Pera' orange in 1978

Rootstocks	Bearing Dens. (kg/m <sup>3</sup> )	Fruit Wt. (grams)	T.S.S. (%)	T.A. (%)	Brix/Acid ratio
Rough lemon	1.88 ns <sup>Z</sup>	181.6 cd <sup>Y</sup>	9.50 ns <sup>Z</sup>	1.27abc <sup>Y</sup>	8.2 cd <sup>Y</sup>
Rangpur	3.21	174.5 cde	10.95	1.25abc	9.0 c
Kona orange	2.96	182.3 cd	10.74	1.34a	8.7 c
<u>C. taiwanica</u>	2.91	158.2 de	10.85	0.86 d	11.3ab
Siameño	2.96	213.2ab	10.20	1.20abc	8.7 c
Troyer	4.49	180.3 cd	10.32	0.90 d	12.9a
Batangas	2.42	202.0abc	10.04	1.17abc	9.6 bc
<u>C. sunki</u>	5.06	197.4abc	11.15	1.29ab	10.0abc
Cleopatra	3.66	187.7 bc	11.30	0.99 cd	12.0a
Heen naran	5.56	217.7a	11.26	1.18abc	10.4

<sup>Y</sup> Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>Z</sup>ns: no significant difference at 5% level.



'Pera' orange was somewhat stunted on C. taiwanica, 'Heen naran', 'Troyer' and 'Siamelo' rootstocks, with unsatisfactory production in 1978.

Scion overgrowth was observed with trees on 'Heen naran', C. sunki, 'Kona orange' and 'Rangpur' rootstocks. Trees on 'Troyer' showed stock overgrowth. Trees on 'Cleopatra', 'Batangas', rough lemon and C. taiwanica rootstocks had relatively smooth graft unions (see Table 8).

### Ortanique

'Ortanique' yielded best on 'Batangas', rough lemon and 'Kona orange' rootstocks in the 1978 harvest season. Rootstocks which appeared to induce medium bearing included 'Cleopatra', C. sunki, 'Rangpur' and 'Heen naran'. Poor production was observed on 'Troyer' and C. taiwanica. 'Ortanique' trees on 'Siamelo' were severely stunted and produced no fruit (see Table 10).

Differences in tree height and volume of tree canopy were not significant between 'Ortanique' trees grafted on rough lemon, 'Rangpur', 'Cleopatra' and Kona orange'.

Citrus sunki is a potentially promising rootstock for 'Ortanique'. Trees of this combination were somewhat dwarfed, with an average height of 2.1 meters and a low spreading canopy of about 5.5 meters. Production for 1978 harvest season was as high on C. sunki as on larger trees on 'Cleopatra' and 'Rangpur' rootstocks (see Table 10).

Relatively smooth graft unions were found on trees grafted on C. sunki, 'Rangpur', 'Kona orange', 'Cleopatra', 'Heen naran' and rough lemon. Trees on 'Batangas', 'Siamelo', 'Troyer' and C. taiwanica showed

Table 10. --Main effects of rootstock on tree height, canopy volume, trunk girth ratio and yield of 'Ortanique' in 1978

Rootstocks	Tree Height (meters)	Canopy Volume (cubic meters)	Trunk Girth ratio	Yield (1978) (kg)
Rough lemon	3.4ab <sup>y</sup>	11.35 b <sup>y</sup>	0.93abc <sup>y</sup>	76.5a <sup>y</sup>
Rangpur	3.4ab	8.90 b	0.95abc	41.5 bc
Kona orange	2.7 bc	7.55 bc	0.94abc	62.3ab
<u>C. taiwanica</u>	2.0 c	2.20 cd	0.72 d	14.1 c
Siameño	1.2 d	0.40 d	0.85 cd	-----
Troyer	2.1 c	3.45 cd	0.80 cd	27.3 c
Batangas	3.8a	17.85a	0.88abc	92.1a
<u>C. sunki</u>	2.1 c	5.45 cd	1.03a	42.4 bc
Cleopatra	3.2ab	9.55 b	0.94abc	43.7 bc
Heen naran	2.1 c	3.22 cd	0.99ab	32.1 bc

<sup>y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

rootstock overgrowth (see Table 10).

'Ortanique' produced the largest fruits on 'Batangas' and rough lemon rootstocks. Smaller 'Ortanique' fruits were produced on 'Troyer' rootstock. Significant differences in bearing density and total soluble solids were not found in 'Ortanique' on the various rootstocks. Lowest acidity and sugar reading were from fruits on trees on rough lemon. A brix/acid ratio of above 10.6 was found from all fruits. Early ripening might be possible through the use of rough lemon and 'Rangpur' since both rootstocks gave a high sugar/acid ratio of more than 14 (see Table 11).

Table 11. --Main effects of rootstock on bearing density of tree canopy, fruit weight, total soluble solids, total acids and brix/acid ratio of 'Ortanique' in 1978

Rootstocks	Bearing Dens. (kg/m <sup>3</sup> )	Fruit Wt. (grams)	T.S.S. (%)	T.A. (%)	Brix/Acid ratio
Rough lemon	6.88 ns <sup>Z</sup>	217.7a <sup>Y</sup>	9.78 ns <sup>Z</sup>	0.76 b <sup>Y</sup>	14.1a <sup>Y</sup>
Rangpur	4.66	211.0ab	10.21	0.87ab	14.5a
Kona orange	9.08	207.9ab	10.15	0.89ab	12.6abc
<u>C. taiwanica</u>	8.30	199.0ab	10.35	0.94ab	11.4 bc
Siameño	-----	-----	-----	-----	-----
Troyer	8.54	190.5 b	10.44	0.90ab	13.2ab
Batangas	5.16	219.2a	10.42	1.01ab	11.3 bc
<u>C. sunki</u>	7.69	204.2ab	10.93	1.09a	11.1 bc
Cleopatra	4.66	203.6ab	10.99	1.07a	11.6 bc
Heen naran	9.80	217.0ab	10.00	1.14a	10.6 c

<sup>Y</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>Z</sup>ns: no significant difference at 5% level.

## V. CONCLUSIONS

Tristeza decline is a wide-spread virus disease of citrus in Hawaii. It proved to be a severe problem with citrus growing in the Malama-Ki Experimental Farm. In this study, one hundred percent natural infection by the tristeza on virus-free nucellar plants occurred within fifteen years after the orchard was planted.

The virus assays with the Immunodiffusion test and the viral inclusions staining with Azure A were accurate and provided a rapid and efficient diagnosis of infected plants.

In this experiment, popular rootstocks such as rough lemon, 'Rangpur', 'Kona orange', and 'Cleopatra' performed well as rootstocks for the four scion cultivars used. As expected, trees on rough lemon outyielded those on most other rootstocks, but induced poor fruit quality. Scion influence were observed on tree size. Tree of 'Ortanique' and 'Valencia' were generally smaller than those of Navel and 'Pera'. Navel orange was graft compatible with all of the rootstocks in this experiment, and the resulting trees with few exceptions, were large and vigorous.

Rootstock influences on tree size were most obvious with 'Batangas' and rough lemon rootstocks. These two rootstocks stimulated vegetative growth resulting in large trees with broad canopies. 'Ortanique' grafted on 'Batangas' produced exceptionally large trees, noticeably different from the compact trees developed on other rootstocks.

'Rangpur' and 'Kona orange' rootstocks induced upright growth habit on certain scion cultivars and might be suitable for use in high density plantings.

Mandarins are potentially good citrus rootstocks. 'Batangas', C. sunki and 'Heen naran' were the mandarin rootstocks tested in this experiment. In general, except with 'Valencia', 'Batangas' performed similar to rough lemon. Trees grafted on this rootstock were vigorous and large. Production was as good as on 'Rangpur', better than on 'Cleopatra' and about equal to rough lemon in various scion/rootstock combinations. Fruit quality was slightly better than on rough lemon, with higher soluble solids and acids. A possible disadvantage in using 'Batangas' may be biennial bearing (Joiner, 1955) and excessive tree size.

'Heen naran' rootstock performed well with Navel orange in this experiment. Production was as good as on 'Cleopatra' with similar tree and fruit characteristics. 'Heen naran' did not, however, perform well in other scion/stock combinations, with trees poorly developed and of low productivity.

Tree size on C. sunki varied significantly with different scion cultivars. Except for Navel orange, trees on this stock were somewhat dwarfed with low spreading canopies. Production was, however good, with yield comparable to that on 'Cleopatra', 'Rangpur', and 'Kona orange'. Fruit size and quality were among the best in the experiment. Due to reduced tree size, trees on C. sunki did not produce as well as those on rough lemon. The reduced tree size associated with C. sunki rootstock would be an advantage for use in back-yard and/or high density plantings. Citrus sunki also has good tristeza tolerance.

Navel orange was medium in productivity on C. taiwanica, 'Siamelo' and 'Troyer', but with 'Pera' and 'Ortanique', production was low on

these rootstocks. 'Siamelo' and 'Troyer' were also poor rootstocks for 'Valencia'.

Citrus amblycarpa, sour orange and 'Sampson' tangelo are highly susceptible to tristeza. For this reason, these rootstocks are not suitable for oranges or mandarins in this area.

'Cleopatra' mandarin and 'Rangpur' are the preferred rootstocks under the condition of this experiment. Both are compatible with most citrus cultivars, tolerant to tristeza virus, more or less tolerant to root rot. They were also relatively productive in this experiment and fruit quality was satisfactory. 'Cleopatra' is considered slightly better than 'Rangpur' in having more compact trees, higher soluble solids and adequate acid content.

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