

**A STUDY OF INTRODUCED CLONES OF SWEET ORANGE (*Citrus sinensis*)
AND POSTHARVEST DEGREENING OF 'VALENCIA LATE' ORANGES IN KENYA**

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Short Title:

**CITRUS CLONES EVALUATION IN KENYA AND POSTHARVEST
DEGREENING.**

ABSTRACT

Paul Kiuru

M.Sc.

PLANT SCIENCE

A STUDY OF INTRODUCED CLONES OF SWEET ORANGE (*Citrus sinensis*) AND POSTHARVEST DEGREENING OF 'VALENCIA LATE' ORANGES IN KENYA

The performance of eleven 'Valencia Late' and nine 'Washington Navel' orange (*Citrus sinensis*) clones all on rough lemon (*Citrus jambhiri*) rootstock was evaluated. Significant differences in trunk cross sectional area, plant canopy volume, cumulative yield and yield efficiency were found between clones of different citrus cultivars. Some clones such as VL106, VL139, VL185 and WN204 appeared to be promising in terms of good growth characteristics and high yield, and could therefore be used for the national performance trials. Studies on post-harvest degreening of 'Valencia Late' oranges were also carried out at Matuga Regional Research Sub-Centre (Kenya) in a series of experiments. Fully mature fruits were dipped for three minutes in 0, 500, 1000, 1500, 2000, 2500 and 3000 ppm concentrations of ethephon. Fruits wrapped in aluminium foil shrivelled less and retained their firmness and freshness. Rind brightness increased by dipping of fruits in ethephon (2000) ppm giving a good colour change. Dipping fruits a second time three days after the first dip did not have any significant effect on colour change. (Chemical names used: (2-Chloroethyl)phosphonic acid (ethephon)).

RÉSUMÉ

M.Sc.

Paul Kiuru

PHYTOTECHNIE

ÉTUDE DE CLÔNES (SCIONS) D'ORANGERS DOUCES INTRODUITS ET DE LA DÉCOLORATION DES ORANGES VERTES DE 'VALENCIA LATE' AU KENYA

Nous avons évalué onze clônes du cultivar (scion) d'oranger 'Valencia Late' et neuf clônes du cultivar (scion) d'oranger 'Washington Navel', tous sur le porte-greffe citronnier à fruits rugueux (*Citrus jambhiri*). Nous avons observé des différences significatives entre les clônes des différents cultivars d'oranger quant à l'aire transversale du tronc, le volume de la voûte de l'arbre, le rendement cumulatif et l'efficacité à rapporter. Quelques clônes tels que VL106, VL139, VL185 et WN204 nous sont apparus promoteurs quant à leurs bonnes caractéristiques de croissance, de haut rendement et pourraient être essayés à l'échelle nationale. Nous avons aussi effectué une série d'expériences sur la décoloration des oranges vertes du cultivar 'Valencia Late' en post-récolte au sous-centre régional de recherche de Matuga (Kenya). Nos traitements ont consisté à tremper des fruits entièrement mûrs pendant trois minutes dans des solutions d'éthéphon de 0, 500, 1000, 1500, 2000, 2500, et 3000 ppm. Nous avons en outre enveloppé des fruits dans une feuille d'aluminium. Ceux-ci se sont desséchés moins rapidement et ont conservé leur fermeté et leur fraîcheur. La coloration orangée de la pelure a augmenté sensiblement suite au trempage des fruits dans l'éthéphon à 2000 ppm. Cependant un deuxième trempage des fruits trois jours plus tard, n'a pas changé

significativement la coloration des fruits. Nom du produit chimique utilisé: acide phosphonique de chloro-2 éthyl.

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My deepest gratitude is expressed to my wife Njoki and my children, Wangu, Mugure and Wambui for their understanding and encouragement during my absence from them during the course of my study.

LIST OF ABBREVIATIONS

avg	average
C	Centigrade
cm	centimetre(s)
cm ²	square centimetre(s)
CGD	citrus greening disease
cum.	cumulative
C.V.	coefficient of variation
diam	diameter
F	Fahrenheit
g	gram(s)
ha	hectare(s)
kg	kilogram(s)
m	metre(s)
m ²	square metre(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
pH	hydrogen ion concentration
ppm	part(s) per million
TA	total (titratable) acid
TCSA	trunk cross-sectional area
TSS	total soluble solids
WN	Washington Navel
wt	weight
VL	Valencia Late
>	more than
<	less than
%	percent(age)
/	per

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CHAPTER 1

1.1 General introduction:

Citrus is one of the major fruit crops in Kenya and is grown mainly for fresh market, a small proportion of citrus crop is used for processing, and a small quantity of lime (*Citrus aurantifolia*) goes to the export market (Seif and Whittle 1984, Anon. 1991). It is grown from sea level (Coast Province) to the highlands of Eastern and Western Kenya ie. at an altitude of 2,000 metres above sea level (Figure 1.1). These growing areas have a diversity of soil types and rainfall with the soils ranging from the Coastal, wind-blown, sandy soils poor in nitrogen and phosphorus to the volcanic loamy soils of Western Kenya. The total rainfall also varies from as low as 900 mm in some parts of Coast province to 1,800 mm in Western Kenya.

The bulk of production is mainly from small scale holdings, where it is grown as an intercrop, and also from a few large scale farms, rather than from large estates. Hectarage of Citrus was estimated to be 20,000 ha in 1981 (Seif and Whittle 1984) and in 1990 hectarage was reported as 18,934 ha (Anon. 1990). Coast province is one of the main citrus growing areas and had 5,544 ha in 1988 which rose to 5,964 ha in 1989 (Anon. 1989a). Eijantten et al. (1980) estimated that of the total hectarage: 70% was sweet orange, 12% lime, 9% lemon, 5% Mandarin and 4% grapefruit.

Among the sweet orange cultivars 'Washington Navel' and

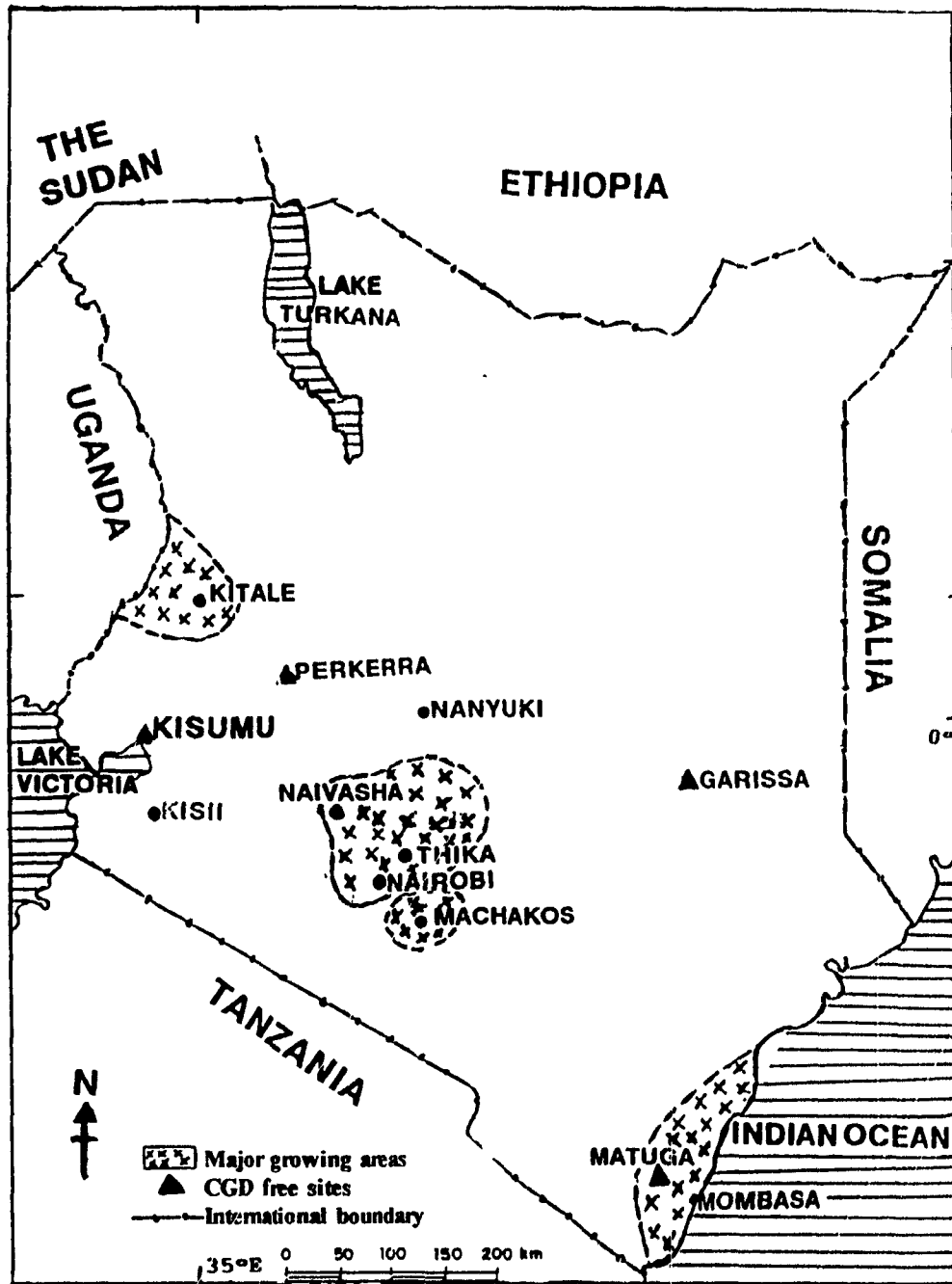


Figure 1.1 Major citrus growing areas in Kenya.

'Valencia Late' are the most important. 'Washington Navel' cultivar grows and yields well at an altitude between 900-2,000 metres and, since growth is regulated by rainfall, a major flush occurs and fruits are ready in 5-6 months after fruit set (Davies 1986). 'Valencia' is a low altitude cultivar and gives the best results between sea level and 1,200 metres. Fruits are smaller than 'Washington Navel' on average but are produced in greater numbers and they remain on the tree several months after ripening.

Citrus production in Kenya has been declining despite new planting and this has been attributed to the high incidence of pests and diseases (Figure 1.2) as well as other factors (Anon. 1991). The demand for citrus budlings has been met by the government and also private commercial nurseries. Scarcity of planting material has brought about a proliferation of citrus nurseries some of which are poorly managed and this adds to the threat of transmission of diseases such as citrus greening and tristeza which lead to declining production. Surveys carried out in 1976 (Schwarz 1976) and between 1981-1983 (Anon 1982, Seif and Whittle 1984) found that the decline in production was due to the high incidence of pests and diseases in the citrus growing areas. Citrus greening disease (Schwarz 1976, Anon. 1982, Seif and Whittle 1984) and *Phytophthora* diseases, gummosis and root-rot (Seif and Whittle 1984) were among the most common. Tristeza disease was found to be the most important among those caused by virus and



Figure 1.2: Typical greening-affected trees; (A) in the field; (B) A tree showing chlorotic foliage and extensive dieback (Thika, Kenya).

virus-like organisms (Seif and Whittle 1984).

The surveys also showed that high quality nursery stock, which was a prerequisite for a successful launching of a citrus rehabilitation programme, was lacking and since a citrus orchard can be no better than the nursery stock will permit; the Kenya government was thus faced with a challenge to develop a successful citrus budwood multiplication scheme to produce horticulturally superior material, free from diseases. High quality disease free scion material was imported from Corsica, France and since citrus varieties may behave quite differently under different climatic conditions, these clones are currently being screened in a mother-block at Matuga, a site free from citrus greening disease. Broadbent (1987) recommends that trees and fruits should be observed over several years for cropping and genetic abnormalities (chimeras) before budwood is supplied from mother-block trees. This mother-block will act as the primary source of disease free planting material to all the nurseries which must be registered and licensed under the Nursery registration programme that was gazetted and became effective on 1st June 1990 (Anon. 1989b).

Kenya's Coast province, one of the main citrus growing areas, is an area with high temperatures, averaging 30°C, and humidity ranging from 65-95%. High temperatures result in fruits with high total soluble solids compared to those grown at higher altitudes but the peel remains green and does not

develop the "typical orange colour" even when the fruit is mature.

In the tropics external colour and internal fruit quality bear little relationship to each other. High temperatures prevent chlorophyll breakdown, a process that requires cool temperatures (Stearns and Young 1942, Young and Erickson 1961) and fruits therefore develop good eating quality while the external colour is green.

Citrus peel colour has an important aesthetic value to consumers who prefer orange coloured fruit and are prepared to pay a premium for them. It is not uncommon for unscrupulous street vendors to sell sour oranges (*C. aurantium*) which attain an orange skin colour in Kenya's Coast Province to unsuspecting customers, who mistake them for sweet oranges (*C. sinensis*). Mature fruits with green peel are viewed with suspicion by buyers who question their maturity. Green peel colour is associated with fruit immaturity in the minds of consumers (McCornack and Wardowski 1977) and fruit vendors are forced to display fruits cut into two halves to reassure customers of the fruits' maturity. This external fruit colour at maturity is a major quality criterion of consumers' preference based on eye appeal. It can be significantly enhanced by degreening using ethylene and therefore a need for research work on postharvest degreening of citrus fruits in Kenya has been identified (Anon. 1991). With increased citrus production, coupled with degreening of fruits may lead to more

exports to attract much needed foreign exchange to meet the requirements of the expanding population. Exports of other horticultural commodities ranked third in foreign exchange earnings in Kenya in 1992; but at present in the Rutaceae family only small quantities of lime (*C.aurantifolia*) are being exported (Seif and Whittle 1984, Anon. 1991). Green is considered a desirable fruit colour for limes, which the public associates with them (McCornack and Wardowski 1977, Wilson 1983).

Preharvest application of ethephon has been reported to cause premature defoliation (Cooper et al. 1968, Jahn and Young 1972, Young et al. 1974, Anon. 1975, Stewart 1977, Morton et al. 1978, Iwahori and Oohata 1980). While fruit drop was reported by Cooper et al. (1968), Young and Jahn (1972a, 1972b), Young et al. (1974), Iwahori and Oohata (1980). Postharvest application of ethephon in Kenya would avoid leaf abscission and also premature fall of immature fruits associated with preharvest applications.

Ethephon is a liquid which breaks down to release ethylene. It can be applied without the use of confining gas chambers i.e. air tight degreening rooms (Bondad 1972, Jahn 1973). This would be cheaper, since construction of these chambers is an expensive investment, outside the reach of most Kenyan citrus producers.

1.2 Objectives of these studies:

1.2.1 Citrus clone evaluation:

The objectives of this study were to screen citrus clones being grown in the country for the first time in terms of their growth, yield and also fruit quality.

Trees were also inspected to eliminate clones that were diseased or had poor horticultural characteristics. In this field evaluation, factors considered were: tree size, fruit yield, size, quality, off-type fruits and the trueness-to-type of the new introductions. The purpose of the study was to aid us in selecting clones to be used in the national clonal performance trials which will provide farmers with disease free high quality planting material. This clean stock is useful in limiting or preventing damage from vector transmitted diseases in areas where natural spread is slow or non-existent (Calavan et al. 1978).

1.2.2 Postharvest degreening of citrus fruits:

Conflicting data exist from many other parts of the world mainly because of the well-known effects of local climatic factors on the degreening process but also because of differences in application procedures when ethephon is used as the degreening agent. The aims of this study were:

to evaluate the possible use of ethephon (2-chloroethyl phosphonic acid) as an agent for citrus fruit degreening in Kenya.

to establish the ideal rates for optimum postharvest

degreening in Kenyan conditions;

to establish whether fruit response is influenced by the number of dips in ethephon;

to establish whether or not its response is influenced by light;

to assess the postharvest storage losses resulting from the ethephon treatments.

CHAPTER 2

2. Literature review:

2.1 Citrus clone evaluation:

In an effort to improve the quality of nursery stock, fast acquisition of virus-free material for establishment of mother-blocks was possible through importation of budwood and rootstock seed from either Willits and Newcombe, California or the Station de Recherches Agronomique, Corsica, France. Both sources send virus-free material all over the world and also give advice on choice of varieties (Schwarz 1976).

Recommendations for establishment of mother-blocks required that siting was in low lying disease / psylla free areas and the Nursery registration act made it mandatory for citrus nurseries to be only in disease-free areas.

Under the tree nursery registration programme, no person is allowed to establish or operate a fruit nursery as from 1st June 1990 unless he/she possesses a certificate of registration. For registration a horticultural inspector has to visit a nursery site and certify it as disease free and suitable for a tree nursery. The inspector also has to be satisfied that the planter or his/her agent has adequate knowledge of nursery management or horticultural techniques in respect of fruit trees to be grown. Rootstock seeds must come from approved sources while the scion material is procured from mother-tree blocks approved by the government. Inspection of nursery material is done in the seed-bed before budding

with a final inspection being done before budlings are approved for sale with minimum nursery standards set by a Fruit Nursery Committee being maintained throughout the growing time. A movement permit issued by Agricultural Office is mandatory for transferring fruit trees from one part of the country to another (Anon. 1989b).

Adequate evaluation of genetic characters of a new variety is said to require observations in various environments over a period of time (Cameron and Frost 1968). Broadbent (1987) recommended that trees and fruits should be observed over several years for cropping and genetic abnormalities (chimeras) before budwood is supplied from mother-block trees. Several years of observations on the imported clones will therefore be needed before recommending their use in propagation nurseries.

2.2 Citrus greening disease:

Citrus greening disease is known to occur widely in sub-Saharan Africa and has been reported in Burundi, Cameroon, Ethiopia, Malawi and Rwanda in addition to the earlier reported cases in Kenya, Mauritius, South Africa, and the Reunion Islands (Aubert *et al.* 1987). Swai (1988), Evers and Grisoni (1991) have also reported the presence of the citrus greening disease in some parts of Tanzania.

Citrus greening disease (CGD) is caused by a phloem-restricted bacterium-like organism (Villeanoux *et al.* (1992). It is one of the most serious and devastating of all

known citrus diseases (Bove 1986, Roistacher 1991) and was first described in South Africa in the early 1930's (Schwarz and Knorr 1973).

In Africa CGD is naturally disseminated by citrus psylla (*Trioza erythrae* Del Guercio). Both the vector and causal organism are intolerant of hot or dry climates (Green and Catling 1971, Bove et al. 1974). The African CGD organism is reported to be sensitive to heat (Bove 1986) and symptoms do not occur in plants grown at temperatures above 32° C; no organisms are found in such symptomless trees. In Kenya CGD has been reported to be wide-spread and severe in cool, elevated areas over 800 metres above sea level but absent from the Coastal region (Schwarz 1976, Anon. 1982, Seif and Whittle 1984, Bove 1986).

CGD is characterised by chlorosis of the leaves (leaf mottle) on one or more limbs, yellow veins, followed by twig die-back, sparse foliage and distinct yellow shoots. Fruits do not fully colour at the styler end and remain green and hence the name "greening" (Wallace 1978, Roistacher 1991).

Fruits from trees affected by CGD are reported to be under-developed, lopsided and poorly coloured and seeds are often aborted (Schwarz 1976). Wallace (1978) reported that most seeds from diseased trees remain small and are dark coloured. The juice is of poor quality, bitter, with unpleasant taste, low in soluble solids and high in acids

(Schwarz 1976, Wallace 1978).

In the late stages the trees are reported to have "open and sparse" growth, chlorotic foliage and show extensive dieback (Figure 1.2). The fruits remain small and drop from the trees prematurely (Schwarz 1976, Wallace 1978, Roistacher 1991). These symptoms in many cases result in a decline in yield and finally death of the trees. CGD has a great socio-economic impact; Aubert (1987) reported that one million trees were lost to the disease in the south eastern part of Africa and that 70% of disease-free material planted in Reunion islands became unproductive six years after planting. In Kenya Seif and Whittle (1984) reported that a total loss of marketable fruit yield was observed in some areas three years after the first symptoms were noticed and infection rates of as high as 100% were found in some old orchards.

2.3 Disease control:

In a study on transmission of CGD Marais and Rea (1985) found that bud and bark inoculation respectively resulted in 65% and 30% transmission of the disease in trees. Distribution of nursery trees or budwood from greening affected areas should not be done since this has been reported to be one of the major factors affecting spread of the disease and its vector to new areas (Marais and Rea 1985, Bove, 1986 and, Aubert 1987).

Other measures have been tried in different areas to control CGD. Tetracycline injections repeated at regular

intervals of one, two or three years have been tried in South Africa with some degree of success (Bove 1986).

In the Reunion islands biological control of the psyllid vector by the introduction of insect parasites *Tetrastichus radiatus* and *T. dryi* for the vectors *Diaphorina citri* and *Trioza erytreae* respectively is reported to have been successful (Aubert and Quilici 1984, Bove 1986). The success of this biological control is reported (Bove 1986) to be largely due to the fact that the parasitic flies were introduced without their own insect parasites (hyperparasites).

Chemical control of the insect vectors and also long term breeding programs for disease resistance using the West Indian lime (*Citrus aurantifolia*) have been reported in South Africa (Bove 1986). Lime trees infected by CGD have been observed to show much greater tolerance compared to sweet-orange (*Citrus sinensis*) and mandarin (*C. reticulata*) trees (Bove 1986., Aubert 1987). Aubert (1987) has reported that breeding programmes using backcrosses with trifoliolate orange seem to give promising results at least in the case of the African greening.

In Indonesia integrated control measures for citrus vein phloem discoloration (CVPD), another name for citrus greening disease, comprises removal of inoculum infected sources, use of disease free planting material and control of the disease vector (Kusumo 1990). In Kenya control of CGD is mainly

through reduction of inoculum levels in the orchards, control of the vector and improvements in nursery management through the establishment of mother-blocks (Figure 2.1) and the nursery registration programme (Seif and Whittle 1984).

2.4 Postharvest degreening of citrus fruits:

Citrus fruits are different from climacteric fruits in that they produce only a trace amount of postharvest ethylene and there is no associated rise in respiration rate (Hyodo 1981). They contain no starch and unlike bananas they cannot be picked green for after-ripening during shipping and storage. Once an orange is picked, it is as good as it will ever be, though the taste becomes slightly sweeter in holding, because its acid is broken down faster than sugar (Samson 1986). Degreening of citrus fruits has therefore been defined by Miller et al. (1940) as a process whereby the fruit loses the green pigment (chlorophyll) whether this takes place naturally on the tree or is hastened by the use of ethylene.

In the citrus growing areas of the humid tropics and warm sub-tropics it is not uncommon to find mature fruits which have a green peel (Soule and Grierson 1986a). This external colour is a function of the climatic conditions in the region rather than internal fruit maturity but this can reduce the marketability of the fruit, since it might wrongly be associated with immaturity, (Grierson et al. 1986).

In the tropics the green peel colour is caused by high temperatures which prevent the breakdown of chlorophyll.

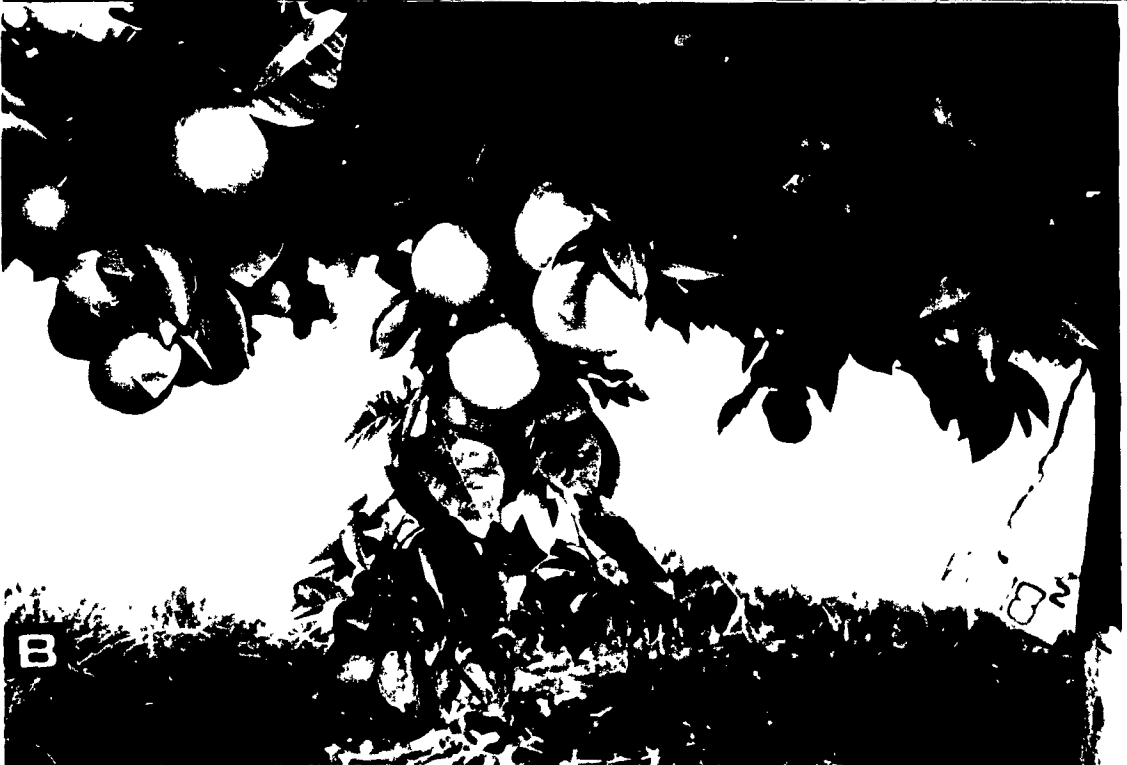


Figure 2.1: (A) A citrus mother-block established in a greening disease-free area in the Coast Province, Kenya; note the wide spacing used between the trees, (B) Fruits from a 'Valencia Late' clone.

Chloroplasts remain in the flavedo throughout fruit development, in contrast to cooler areas where chlorophyll disappears and chloroplasts are converted to chromoplasts (Coggins and Jones 1977). In areas where there are cool night temperatures there is typically a loss of chlorophyll and an increase of carotenoids as fruit matures (Miller et al. 1940). Low night temperatures cause stress that stimulates production of endogenous ethylene (Cooper et al. 1969, Grierson et al. 1986). This internal production of ethylene in stressed fruits is in quantities large enough to destroy chlorophyll in flavedo and thus enhance 'typical varietal colour' development (Grierson and Ting 1978, Grierson et al. 1982). Vine et al. (1968) reported that only traces of ethylene are found in the internal atmosphere of unstressed fruits.

2.5 Historical background of degreening:

The earliest techniques used for degreening citrus employed emanations from ripe fruits or the smoke from burning combustible products.

In an early practice to deliberately wilt the peels of citrus fruits (i.e., 'curing', 'quailing', or 'sweating') in order to harden them, it was believed that the high temperatures and humidity in the rooms caused the degreening. Sievers and True (1912) were the first to demonstrate that degreening was mainly caused by gaseous combustion products

from kerosene stoves with heat and humidity being of minor importance.

In their work Sievers and True (1912), pointed out that exhaust gas ('pungent gas') from automobile engines could colour lemons and it was Denny (1924), who provided convincing evidence that ethylene was the effective constituent of 'stove' gas. Denny found that ethylene, even at very low concentrations caused green lemons to turn yellow and such fruits did not differ from similar fruits coloured using kerosene stoves; but he was unsuccessful in his attempts to isolate ethylene.

Denny (1924) found that the absence of oxygen prevented the coloration of fruits by use of ethylene and that degreening using either ethylene or stove gas increased the rate of respiration of lemons. In his work he noted that coloration was not measurably hastened by ethylene at a temperatures of 45°F (7°C), but the rate of colouring increased with increasing temperature from 57°F (14°C), to 82°F (28°C) with a reduction in the rate being observed at 93°F (34°C).

Thus Denny's work is said to have marked the beginning of the knowledgeable use of ethylene gas in the commercial degreening and fruit ripening process (Sherman 1985).

2.6 Citrus degreening agents:

2.6.1 Ethylene :

Ethylene is a plant hormone which regulates many aspects of growth, development and senescence. Its benefits or harm in horticulture depends on when and where it occurs, (Yang 1987). It is produced either as a response to stress (Vine et al. 1968, Cooper et al. 1969, Grierson et al. 1986) or as part of the normal process of maturation of climacteric fruits (Grierson et al. 1986).

In citrus fruits ethylene is produced under conditions of mild stress i.e., when night temperatures fall below 12.5°C the fruits degreen naturally . This is analogous to the process that is induced by postharvest degreening using exogenous ethylene (Grierson et al. 1986).

The activity of the chloroplastic enzyme chlorophyllase which is thought to play a role in chlorophyll degradation has been shown to increase dramatically in citrus fruit peel in response to ethylene (Barmore 1975, Shimokawa et al. 1978, Purvis and Barmore 1981).

2.6.2 Sources of ethylene:

Ethylene for postharvest degreening is available in various forms:

2.6.2.1 Liquid ethylene:

These are the ethylene releasing chemicals such as 2-

chloroethylphosphonic acid commonly known as ethephon or ethrel®. The chemical rapidly breaks down in water at neutral or alkaline pH values to form ethylene (Warner and Leopold 1969, Salisbury and Ross 1992). The chemical has been used widely in postharvest applications in citrus degreening work (Fuchs and Cohen 1969, Jahn 1976, El-Zeftawi and Garrett 1978, Torres and Pividal 1982). In its use on oranges, Fuchs and Cohen (1969) reported that a treatment with 1000 ppm ethrel turned fruits to a marketable colour after 7 days while high concentrations of ethrel (Fuchs and Cohen 1969, Jahn 1973) not only delayed degreening in oranges, tangerines and lemons but also caused rind injury on lemon.

Abbas *et al.* (1984) reported that oranges dipped in 1000 and 2000 ppm ethephon attained a marketable yellow colour in ten and five days respectively whereas control fruits were still only light yellow 20 days later. These results were in agreement with those of Fuch and Cohen (1969) and, Vakis (1975). Arora *et al.* (1973) working on 'Valencia' fruits, found that a better colour development was achieved with 1000 ppm ethephon within seven days compared with the other concentrations. Further colour development was observed up to 14 days after treatment and thereafter no difference could be observed.

A correlation between the concentration of ethrel applied and colour development was reported on grapefruit (Minessey *et al.* 1972), on 'Washington Navel' (Chauhan and Rana 1974, and

El Hammady *et al.* 1974) and on lemon (Josan *et al.* 1981). They all found that with any increase in concentration of ethrel there was an appreciable degradation of chlorophyll and production of brighter yellow colour compared with the untreated control; Gilfillan and Lowe (1985) however reported that a packhouse dip in 2000 ppm ethephon was ineffective in 'Satsuma' mandarins. Minessey *et al.* (1972) working on grapefruit reported that degreening rate was a function not only of the ethrel concentration but also the length of the post-application storage period.

Preharvest double application of ethephon on the tree has been reported to significantly reduce chlorophyll levels more than single sprays (Young and Jahn 1972b). Eaks (1977) reported that 'California Navel' oranges and lemons dipped a second time in 1000 ppm ethephon one or two days after the first dip degreened at about the same rate as ethylene treated fruits. In this study fruits dipped once in 1000 ppm ethephon did not degreen as rapidly as those exposed to ethylene.

Commercial use of post-harvest ethephon dips for oranges have been reported in South Africa (Gilfillan 1982 cited by Wilson 1983) apparently in lieu of gaseous ethylene treatment.

2.6.2.2 Ethylene gas cylinders:

Cylinders or cans of pure compressed ethylene or ethylene diluted with an inert gas are available (Kitagawa *et al.* 1977,

and Sherman 1985). Molecular sieves that contain 5% ethylene and release it easily in water have been used for Satsuma mandarin (*C. unshiu* Marc.) degreening in Japan (Kitagawa et al. 1977).

2.7 Citrus fruit colour:

2.7.1 Biochemical and physiological changes:

The typical events that occur when citrus fruits change colour are said to be the consequence of chlorophyll degradation and carotenoid build up (Miller et al. 1940). Grierson et al. (1960) reported that in the absence of chlorophyll, the total amount of yellow and red carotenoids and their relative proportions determine fruit colour.

The degradation of chlorophyll and increase or unmasking of carotenoid pigments that are associated with the citrus degreening process have been a subject of study by many authors (Grierson et al. 1972, Stewart and Wheaton 1972, Eaks 1977, Jorgesen 1977).

Although there is agreement that chlorophyll and carotenoid pigments are involved in fruit colour change there are various hypotheses to explain how this colour change is affected by ethylene. Jorgesen (1977) reported that synthetic degreening involves chlorophyll destruction which allows the yellow citrus carotenoid to show up. Kitagawa et al. (1977) working on 'Satsuma' mandarin reported similar results. Cuesta et al. (1981) made similar observations and reported that as fruit reached maturity chlorophyll levels declined drastically

while the carotenoid levels remained constant.

In their study Young and Jahn (1972a) reported that low temperatures and ethylene stimulated the chlorophyll destruction and increased carotenoid levels. They also reported that carotenoid levels continued to increase after chlorophyll destruction.

2.7.2 Environmental factors affecting citrus degreening:

2.7.2.1 Temperature:

Temperature during fruit maturation is known to have an effect on the 'typical orange colour' development of the fruit.

In a study of citrus colouring of several citrus varieties, Stearns and Young (1942) concluded that an exposure to a minimum temperature of below 55°F (13°C) was required to induce a natural colour break. Their work showed that the most important factor related to colour breakdown in citrus peel was cool temperature. In a similar study Young and Erickson (1961) found that a temperature combination of 20°C for daytime air, 7° C for night time air and 12°C for soil produced brightly coloured 'Valencia' orange fruits. This temperature combination resulted in the greatest reduction in chlorophyll and greatest carotenoid increase. Eaks and Dawson (1979) working on the effect of vegetative cover in citrus orchards found that carotenoid content was higher and chlorophyll content lower in the rind of the fruit from plots with a

vegetative ground cover than those from bare ground plots. The ground cover reduced the soil temperature and therefore favoured fruit colour development.

2.7.2.2 Light:

Studies of the effect of light on ethylene treated fruit has given contrasting results.

In a study by Obervacher et al. (1968) it was found that degreening of 'Valencia' orange was somewhat slower in the dark than in the light at 85°F (30°C) when ethephon was used. El-Zeftawi et al., (1978) reported similar results with 'Valencia' orange. In their work fruits were dipped for one minute in ethephon while on the tree. After drying, half the fruits were wrapped in aluminium foil to exclude light. Results showed that in light ethephon increased carotenoid as fruit re-greened but at green and coloured stages chlorophyll levels were slightly decreased. When light was excluded carotenoid levels were greatly depressed in green fruit but only slightly in coloured fruits. Huff (1983) found that regreening of Citrus epicarp *in vitro* required light and none occurred in the dark though degreening occurred both in the dark and light.

Chauhan and Parmar (1972) reported different results, in their work on 'Mosambi' orange they found that fruits treated and stored in the dark were brighter yellow and glossier than those kept in the light.

Arora et al. (1973) observed that 'Valencia' sweet orange

fruits treated and kept in darkness showed neither any remarkable difference in colour development nor in quality, except for the fact that they looked slightly more fresh than those kept in the light. Rana and Chauhan (1976) noted that chlorophyll loss on "Kagzi Lime" fruits dipped in 0-400 ppm ethephon and held for 12 days was not affected by light regime.

2.7.3 Fruit losses associated with post-harvest degreening:

Ethylene gas promotes senescence (physiological aging) Grierson et al., (1986) and thus inevitably increases fruit susceptibility to loss through various physiological and pathological causes.

2.7.3.1 Stem-end rot:

The fungi causing this post-harvest fruit decay are always present in the orchard but do not usually affect fruit until after it is picked (Salunkhe 1984, Tusset et al. 1988).

The exposing of citrus fruits to ethylene has been reported to increase the incidence of stem-end rot caused by *Diplodia natalensis* (Stevenson 1954, Grierson and Newhall 1960, Hall et al. 1960, Winston 1960, Jahn et al. 1969, Jahn 1973 and, Cohen 1978). Various theories to explain the link between fruit decay and degreening have been put forward. A rapid abscission of "buttons" i.e., calyx resulting from ethylene degreening is reported (Brown and Wilson 1968, McCornack and Wardowski 1977) to leave microscopic openings for stem-end rot organisms to grow into the fruit. High

ethylene concentration is reported to promote the germination of such spores on the flavedo (Abeles 1973) and thus cause the rot. Brown (1986) however associated this increased stem-end rot associated with elevated ethylene rates not with more rapid abscission of buttons or to stimulated activity of quiescent infections on button tissue but what he thought of as ethylene action in enhancing the growth rate of fungus or predisposing cells of the abscission zone to hyphid penetration.

McCornack (1971) working on Florida citrus, Brown and Barmore (1976) on tangerines and Cohen (1978) on 'Shamouti orange found that in a comparison between non-degreened fruit and fruits degreened with ethylene there was a direct relationship between ethylene concentration applied and decay. Fruits treated with ethylene during postharvest degreening had a greater amount of decay. Increasing the ethylene concentration during degreening resulted in increased fruit decay after picking. Cohen (1978) also reported that the incidence of stem-end rot which developed during degreening was also influenced by the duration of treatment. Fruit losses through decay were found by Jahn *et al.* (1969) to be greatest at higher ethephon concentrations and temperatures, but were less and occurred later than in gaseous ethylene degreening. Contrasting results have been reported on grapefruit, where Hatton and Cubbedge (1973) found that ethylene had no significant effects on aging and decay while El Hammady *et al.*

(1974) reported similar results with 'Washington Navel' oranges where they found no indication that ethephon treatment caused any increase in fruit rot.

2.7.3.2 Fruit staining:

Dark fruit staining on the flavedo during fruit curing were reported to be caused by ethylene gas (Ben-Yehoshua et al. 1990). Hatton and Cubbedge (1973) found that there was rind injury on grapefruits exposed to higher ethylene concentration for long periods.

2.7.3.4 Anthracnose:

Brown and Barmore (1976) postulated that the anthracnose fungus survives on fruits in the form of appressoria which, in most instances, do not produce infectious hyphae until stimulated by ethylene.

Rind damage is caused by *Colletotrichum gloeosporioides* and is said to be common when the latency of infection is broken by high concentrations of ethylene (Wild 1990).

CHAPTER 3

3 Materials and methods:

The studies were carried out at the Matuga Centre of the Kenya Agricultural Research Institute. The Centre is in Kenya's Coast Province and lies on latitude 4° 12' South and longitude 39° 48' East at an altitude of 120 Metres above sea level.

The site has wind blown sandy soils poor in nitrogen and phosphorus. The rainfall is bimodal i.e., has two peaks April to July (this being the long rain season) and September to November (short rain season) and the annual rainfall is 1100 mm. The temperatures are generally high, averaging 30° C with humidity ranging from 65-95% (Ouko and Abubaker 1988).

3.1 Citrus clone evaluation :

3.1.1 Plant material:

The citrus clones were imported as budwood in 1983 from the French Citrus Improvement Program - Corsica , France. This French program was initiated in 1959 in order to supply horticulturally superior budwood known to be free from virus-like diseases. A wide range of species and varieties from the Mediterranean Basin or North America were submitted to tight quarantine and indexing program and candidate plants that passed were established in a foundation block at Corsica (Chabrier and Caruana 1992). In the program various methods were used to ensure that materials were disease free and among them were the production of nucellar plants, thermotherapy and

shoot-tip micrografting. The methods when used singly or in combination, have proved effective in eliminating virus and virus-like diseases, and therefore enabling the reutilization of valuable, sanitized resources. These clones were originally selected as seedling progeny or graft of 'Washington Navel' or 'Valencia Late' and minor genetic difference exist between them because of use of seed as a source of plants and the diversity of regions of origin and plant material used (appendix A). The buds were T-budded on rough lemon rootstock (*Citrus jambhiri*) in a field nursery. Rough lemon as a rootstock for citrus produces fruits that are low in total soluble solids, acid, and ascorbic acid content (Davies 1986). At the time of budding budwood and budding knives were disinfected by immersion in a sodium hypochlorite solution. The young trees were maintained in the nursery for about two years.

3.1.2 Planting:

Planting was done during the rainy season in 1985. The soil around the plant was thoroughly wetted and irrigation was used in the dry days that followed. As a rule of thumb Samson (1986) recommends that 10 litres of water per plant is needed once a week.

Planting holes were dug 60 cm x 60 cm wide and 60 cm deep. Triple super-phosphate at the rate of 100 grams per tree was applied. Organic manure was mixed with top-soil and was used for infilling the planting holes. Trees were planted

at a spacing of 9 m X 9 m , (123 trees/ha) instead of the recommended spacing of 6 m X 6 m , (278 trees/ha) to avoid the risk of accidental root grafting between adjacent trees. Bintacourt and Fawcett (1944) reported that psorosis disease could be transmitted from one tree to another through root grafting which is common in citrus. This wide spacing (Figure 2.1) also makes it easier to uproot a clone with minimal disturbance to those left in the field if it is found to be diseased .

A basin was formed around the tree by clean weeding and raising the soil embankment. The diameter of the basin was determined by the spread of the leaf canopy and generally its diameter was an average of the measurement of East-West and North-South canopy widths (Figure 3.1). Grass mulch cover was put in this basin to reduce water loss from the soil. Irrigation water was normally directed into this basin by use of a hose pipe and the basin was useful in ensuring that the irrigation water was held around the root zone. Irrigation was stopped when the soil in the basin reached its field capacity.

3.1.3 Cultural practices:

Annual application of fertilizers and farm-yard manure followed standard recommendations. Plants were top-dressed with nitrogen fertilizer twice a year to coincide with the onset of the two rainy seasons. Routine pest and disease control was done according to standard practices.

The volunteer grass sod between and within rows was



Figure 3.1: Measurement of plant canopy width and height. Grass mulch is in the basin around the tree.

regularly mowed with a tractor drawn mower. Grass mulch was applied around the base of each tree which was also kept free of weeds by regular hand weeding. Coconut leaves were used to provide shade for the first few months after transplanting.

3.1.4 Citrus clones in the experimental plots:- There were twenty citrus clones represented in the two experimental plots of which eleven were 'Valencia Late' and nine 'Washington Navel':

'Valencia Late' (VL) clone:

VL 18	VL 35	VL 53	VL 104	VL 105
VL 106	VL 107	VL 139	VL 185	VL 247
VL 248				

'Washington Navel' (WN) clones:

WN 39	WN 102	WN 135	WN 141	WN203
WN 204	WN 205	WN 215	WN 216	

The original plan for the mother block was not well designed and it was not laid out in a way which would facilitate future analysis. Thus only by making assumptions about the uniformity of the plot was it possible to undertake a statistical analysis of the data on tree performance for this study. Dr. P. Dutilleul, Statistician assisted with this and suggested the procedure to adjust for rows and columns effects as covariates in the analysis of the data but both covariates were non-significant. Separate adjustment for either row or column as a covariate led also to the non-significant effect of the covariate leading to the analysis as

a completely randomized design.

3.1.5 Growth parameters:

Growth was measured annually during the month of May (Figure 3.1). Three measurements of tree size were taken to give an estimate of the relative vigour of the clones.

Trunk diameter (cm): was measured at 5 cm above the bud graft union in centimetres. It gives an indication of the annual rate of tree growth and can be influenced by cultivar, yield, virus diseases and pests, soil moisture and cultural factors (Reuther 1973).

Tree height (m): Height was estimated by measuring from ground level to the top-most shoots.

Canopy spread (width): Width was a mean of the canopy spread taken in the East-West and North-South directions measured at approximately one metre above ground level. The mean was the average of the two measurements taken at right angles.

Canopy volume: This was calculated according to the method of Wutscher and Shull (1972, 1978) and Hutchison (1977),

$$\text{Canopy volume} = (\text{Width}^2 * \text{tree height}) / 4$$

3.1.6 Yield :

Fruit yields were recorded at the time of each harvest. Mean number of fruits per tree and mean fruit weight per tree were both recorded. The counting of the fruits in yield

determination gave a measure of the crop which was potentially available.

Cumulative fruit yield data over a number of years were used, to reduce the effect of yield fluctuations from season to season and also to take into account variability in yield due to initial juvenility of the trees. Yield variations are higher in the first 5-6 years of a tree's life due to pronounced juvenile characteristics in the scion, such as thorniness, rank (excessive) vegetative growth, a reluctance to flower and set fruit, and alternate bearing which is common when budding is done on a seedling rootstock (Cameron and Soost 1969., Cary and Weerts 1977).

3.1.7 Fruit quality :

Citrus growers are interested in higher yields, consumers in desirable fruit size and fruit quality. A maximum combination of all these characteristics should be the ideal to strive for in selecting new parent trees.

Fruit quality was determined by physical and chemical means. Fruits were always picked when mature and quality was determined from a composite sample of 10-20 fruits per clone.

Fruit diameter and height (length): were determined from a random sample of 10-20 fruits. Fruit diameter (cm) was measured across the fruit at right angles to the axis i.e., around the equatorial section of the fruit. Fruit height (length) was measured along the axis from stem to stylar end (apex) in centimetres.

The fruit diameter and height indices were obtained by totalling the indices of all the individual fruits measured and dividing by the number of fruits. These indices are used in the description of the fruits (external characteristics) and the ratio of diameter to height was used as an indication of fruit shape. A value of near 1.0 indicates that the fruit is essentially round. Higher values indicate a flatter shape while lower values indicate a more elongated shape (Jahn 1970).

Peel (rind) thickness was measured in mm. The peel thickness was measured at the equatorial section of each fruit by cutting open the fruits.

Mean fruit weight in grams was also recorded.

3.1.8 Fruit juice analysis:

Citrus fruits contain no starch, unlike bananas, and thus cannot be picked green for after-ripening. As long as the fruit hangs on the tree, the Brix continues to rise, at first quickly, then gradually slower, it may go up to 13° or even higher (Samson 1986).

3.1.8.1 Total soluble solids (TSS): Soule and Grierson (1986b) reported that about 75-80% of the TSS in the juice of non-acid citrus is sugar, principally sucrose and glucose plus fructose in roughly equal amounts. This proportion is high enough and sufficiently consistent that TSS or °Brix (a processing term widely used for fresh fruit) can be expressed in terms of percentage pure sucrose (Soule and Grierson

1986b). °Brix (degrees Brix) is a term used to designate the percent by weight of dissolved sugar in a solution and in citrus is one of the most important determinants of quality. The °Brix of a juice is used as a factor in determining maturity of fruit McAllister (1980). TSS is determined by either the measuring the specific gravity with a hydrometer, or the refractive index with a refractometer, Soule and Grierson (1986b).

Fruit juice was extracted from a random sample of fruits using a hand juice extractor. The total soluble solids were determined by use of a refractometer. This gives % by weight of dissolved sugars in a solution and it indicates the % of soluble solids in the juice. In this method of AOAC (1984) concentration of sucrose has the same refractive index as solution being analyzed.

3.1.8.2 Total acidity determination (TA): Citric acid is the main organic acid present in citrus fruits. It was determined according to the methods described by AOAC (1980). The acid content is determined as the total titratable acid calculated as anhydrous citric acid and then expressed as "percent by weight" (grams per 100 grams of juice). TA is measured by titrating a known volume of juice with standard sodium hydroxide (NaOH) and phenolphthalein indicator and then converting the quantity (ml) of alkali to percentage anhydrous citric acid.

In citrus fruits most of the total acidity of the juice

comes from citric acid and consequently the relationship of the percentage of citric acid to TSS (°Brix) is analogous to that of the total acidity (Iranzo and Toran 1977). The °Brix/total acid ratio is used as an index of fruit maturity in most of the citrus growing areas. A ratio is calculated by dividing the °Brix of the juice by percent citric acid that it contains. It is an indication of the relative tartness or sweetness of juice and the lower the ratio the more tart the juice tastes, the higher the sweeter (McAllister, 1980).

3.1.8.3 Vitamin C (ascorbic acid): Citrus fruits have long been noted as excellent sources of dietary vitamin C which is by far the most abundant vitamin in them. The ascorbic acid content of the juice in different citrus fruits varies considerably, and the content will vary with stage of maturity, fruit variety and climate (Nagy 1980). Ascorbic acid concentration is high in immature fruit, and decreases as the fruit ripens and increases in size according to Harding *et al.* (1940). A positive correlation between vitamin C and the soluble solids of the juice of 'Valencia' oranges from the same tree was reported by Sites and Reitz (1951). Vitamin C was determined using standard laboratory procedures.

3.1.9 Preference / acceptance tests:

A ten-point hedonic scale method was used. The essentials of the rating scale method are first the definition of a psychological continuum, and second, the establishment of a series of successive categories of response. In this method an

assumption of a continuum of preferences is made and thus is useful in defining the categories of response (Peryam et al. 1957). In these preference /acceptance tests the personal feeling of a panellist towards a product directs his response and is a measure of preference or a measure from which relative preference can be determined (Larmond 1977).

Random samples of fruits from each clone were evaluated by a consumer-type sensory panel made up of at least ten diverse Centre's personnel with a willingness to participate. Typically samples were identified by a number or letter code which the subject wrote on a score card according to the method of Peryam et al. (1957). Instructions designed to suggest the continuum in order to make the subjects task simple were given. The subjects were requested to give an honest opinion and in this method they are reminded that they are the judge (Peryam et al. 1957). Fruits samples were graded on a 10 point scale ranging from "very poor" for a score of one to a high of "very good" which was accorded a score of ten. In the preference / acceptance tests visual appearance was also important with the fruit size, dimension and weight playing an important role.

Fruit shape and form, skin smoothness, colour and absence of external or internal defects were important considerations. Physical, mechanical and also physiological, pathological or entomological defects also determined the scores.

Fruit firmness, hardness, softness, succulence and

juiciness were important components of fruit texture. Sweetness, sourness (acidity), bitterness, aroma, off-flavour and off-odour were considered as important components of fruit juice flavour which comprises both taste and smell.

The data obtained were analyzed according to the Statistical Analysis System (SAS Institute, 1985). An F-test was used to determine the significance of variation caused by different treatments i.e., clones (Steel and Torrie, 1980). Duncan's Multiple Range Test at 0.05 level of significance was used to compare the differences among the clones.

3.2 Postharvest degreening of citrus fruits:

These studies were carried out during the period May to August 1992. Ethephon, (2-chloroethylphosphonic acid) was used as a source of ethylene gas in a series of experiments to determine its usefulness for fruit degreening. Seven rates were used i.e., 0, 500, 1000, 1500, 2000, 2500 and 3000 ppm.

The cultivar 'Valencia Late' was chosen for these experiments. Fruits were picked and selected to give uniform samples for each experiment in terms of colour, size and ripeness.

Fruit colour was determined before treating with ethephon using a colour chart (Harding and Sunday, 1953). The chart has twelve different colours and numerical values were assigned to each colour. Ten fruits received each treatment. Each fruit was numbered and the colour was determined from the stem end

(top) and also the stylar end (bottom). The mean of these two evaluations was used as the colour score for the fruit. This external colour determination was repeated over a period of time. These visual colour differences are well correlated to the external colour measured with a Hunter colour difference meter (Stewart and Wheaton, 1972).

A periodic assessment of postharvest fruit injury/ losses was made and rotten fruits were recorded and discarded. A count of decayed fruits and also the number of days from picking was recorded.

The data obtained in these series of experiments were analyzed according to the Statistical Analysis System (SAS Institute, 1985). An F-test was used to determine the significance of the variation caused by treatments and their interactions (Steel and Torrie, 1980). A Duncan Multiple Range Test at 0.05 level of significance was carried out to test the differences among the treatments.

3.2.1 Experiment 1: Determination of the influence of Ethephon rates and the number of dips on fruit colour and postharvest decay.

Fruits were picked and selected to give uniform samples in terms of colour, size and equal colour break and free of surface blemishes, injuries and disorders. Initial fruit colour was determined before treating the fruits with ethephon. This colour determination was done from the stem end

(top) and also stylar end (bottom) of each fruit using a colour chart (Harding and Sunday 1953).

Seven ethephon rates were used in this experiment i.e., 0, 500, 1000, 1500, 2000, 2500 and 3000 ppm and for each rate depending on the treatment, fruits were either dipped once or received a second dip three days after the first one. The fruits were dipped for three minutes in an aqueous solution of ethephon soon after colour determination. Excess solution was removed by use of blotting paper. A randomised complete block design replicated four times was used.

Fruits were then kept in open boxes and observations were made at regular intervals throughout storage when changes in colour values were noted. Inspection was also done to determine the extent of decay. Decayed fruit were discarded and sound fruits held. Fruit rot incidence was recorded.

3.2.2 Experiment 2: Determination of ideal ethephon rates for postharvest degreening.

Fruits were picked and selected for uniformity as regards colour, size, freedom from surface blemishes, injuries and disorders. Fruit colour was also determined using a colour chart before treatment with ethephon.

Ethephon was applied at seven rates i.e., 0, 500, 1000, 1500, 2000, 2500 and 3000 ppm. Fruits were dipped in the ethephon solution of required concentration for three minutes and excess solution was removed using a blotting paper. Fruits were then kept in open boxes and observations were made over

a period of time when changes in colour were noted and also the fruit rot incidence was recorded. A randomised complete block design replicated four times was used for this experiment and the experiment was repeated three times commencing on the following dates June 11, July 14 and July 28, 1992.

3.2.3 Experiments 3: Determination of the influence of light on fruit response to ethephon.

Fruits were picked and selected for uniformity as regards colour, size, freedom from surface blemishes, injuries and disorders. The fruit colour was also determined using a colour chart before treatment with ethephon. Seven rates of ethephon 0, 500, 1000, 1500, 2000, 2500 and 3000 ppm. were applied.

Fruits were dipped in ethephon solution for three minutes and excess solution was removed by use of a blotting paper; one lot was kept in light while the other was kept in darkness. Aluminium foil was used to exclude the light. The final fruit colour was recorded using a colour chart. A randomised complete block design replicated four times was used and the experiment was repeated two times commencing on July 14 and July 28, 1992.

CHAPTER 4

4: RESULTS

4.1 Citrus clone evaluation:

4.1.1 'Valencia Late' clones:

4.1.1.1 Growth parameters:

There were significant differences in trunk cross-sectional area (TCSA) among the 'Valencia Late' clones (Table 4.1). VL107 and VL106 had the highest mean TCSA. The clones with the lowest mean TCSA were VL35 and VL105.

Plant height among the 'Valencia Late' clones did not show any significant differences (Figure 4.1) though VL106 plants were tallest and VL105 the shortest. There were significant differences in plant width with VL248 having the greatest and VL35 the smallest (Figure 4.2).

Tree canopy volume also showed significant differences between clones (Table 4.1). VL248 were the most vigorous trees and had the greatest canopy volume although this was not significantly different from VL106. VL35 and VL105 had the smallest canopy volume.

The yield performance of the clones (Table 4.1) was determined by counting and weighing the fruits from each tree annually. Yield varied considerably among the clones and there were significant differences in the cumulative yields in terms of fruit numbers per clone. VL106 had the greatest cumulative fruit yield in terms of fruit numbers and VL18 the smallest. Fruit yield based on weight in kilograms per clone also showed

Table 4.1 Trunk cross-sectional area (TCSA), canopy volume, cumulative fruit yield and cumulative yield efficiency of 'Valencia Late' clones (Means of four observations).

Clone	TCSA (cm ²)	canopy volume (m ³)	cumulative yield (fruits/tree)	cumulative yield (kg/tree)	cumulative yield efficiency ^x	cumulative yield efficiency ^y
VL18	103.58 bcd ^z	10.23 cd	107.5 c	29.75 d	10.41 d	2.87 d
VL35	88.03 d	7.50 d	147.0 c	33.55 d	20.18 cd	4.6 cd
VL53	148.94 a	14.15 abc	653.7 ab	119.85 bc	44.36 abc	8.21 abc
VL104	156.94 a	10.82 bcd	578.0 ab	110.35 bc	54.06 ab	10.24 ab
VL105	97.56 cd	8.28 d	304.8 bc	62.80 cd	32.96 bcd	6.67 bcd
VL106	169.14 a	15.40 a	970.5 a	188.06 a	62.75 a	12.15 a
VL107	172.00 a	14.78 ab	682.2 ab	117.85 bc	45.06 abc	7.83 bc
VL139	139.43 ab	14.08 abc	666.7 ab	149.47 ab	48.09 ab	10.73 ab
VL185	137.44 abc	14.04 bcd	768.0 a	145.37 ab	54.03 ab	10.26 ab
VL247	102.53 bcd	11.06 bcd	351.5 bc	57.20 cd	30.70 bcd	5.07 cd
VL248	150.90 a	16.65 a	643.0 ab	120.60 bc	38.38 abc	7.14 bc
MEAN	132.35	12.45	533.91	103.17	40.09	7.80
C.V	19.38	21.25	44.98	40.28	39.83	33.85

^x cumulative yield efficiency = cumulative fruit yield (fruit number) 1989-91 / canopy volume 1992.

^y cumulative yield efficiency = cumulative fruit yield (kg) 1989-91 / canopy volume 1992.

^z Means within a columns followed by the same letter are not significantly different from each other at P=0.05 (Duncan's multiple range test).

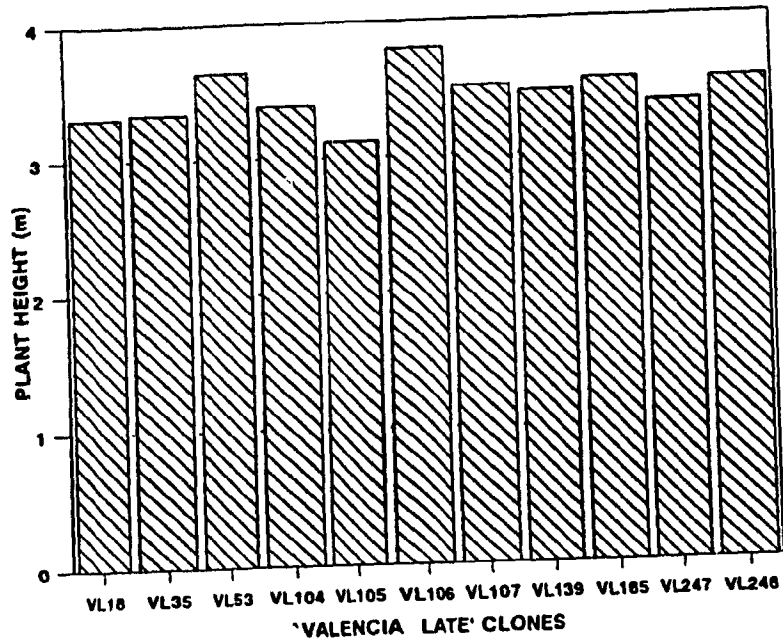


Figure 4.1
Mean plant height of 'Valencia Late' clones (1992).

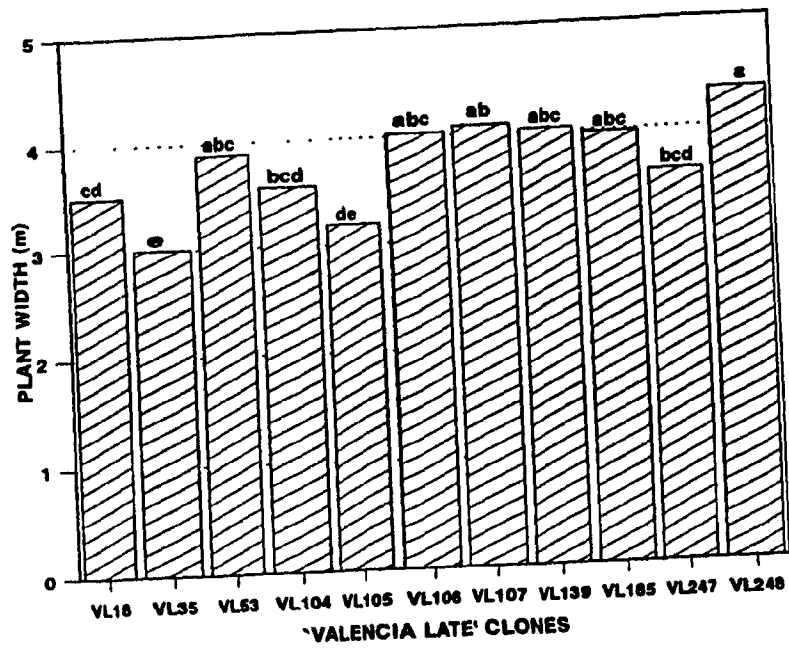


Figure 4.2
Mean plant canopy width of 'Valencia Late' clones (1992).
Bars with the same letter are not significantly different by DMRT at 5% level

significant differences, with VL106 having the greatest weight and VL18 ranking least.

The ratio of cumulative fruit yield to canopy volume is termed yield efficiency. This was expressed based on fruit number or kilograms per clone, it is the most important estimate of a tree's productivity; and was found to be significantly different (Table 4.1). VL106 was most efficient while VL18 was least cumulatively efficient based on fruit numbers. When the efficiency was based on fruit weight in kilograms results were the same, VL106 still had the highest efficiency while VL18 had the lowest.

Correlations between TCSA and cumulative yield both in fruit number and fruit weight were significant with respective $r=0.86$, $P=0.01$ and $r=0.78$, $P=0.01$ (Figures 4.3 and 4.4). A positive correlation coefficient was also found between the canopy volume and cumulative yield both in fruit numbers and weight. This was respectively $r=0.75$, $P=0.01$ and $r=0.77$, $P=0.01$ (Figures 4.5 and 4.6).

The correlation coefficient of the relationship between canopy volume and TCSA was positive and significant $r=0.80$, $P=0.01$. (Figure 4.7).

4.1.1.2 Fruit quality:

Fruit quality data were not statistically analyzed since fruits were picked from each tree within a clone and mixed to form a composite fruit sample to represent the clone. A fruit sample for analysis was taken from this composite sample.

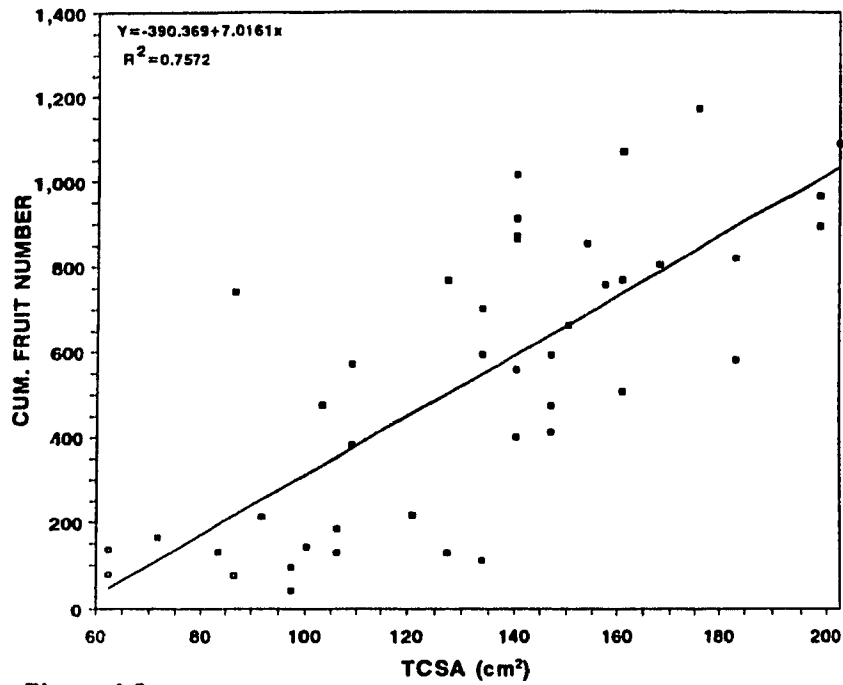


Figure 4.3
Relationship between TCSA 1992 and cumulative yield (fruit number) 1989-91 of 'Valencia Late' trees.

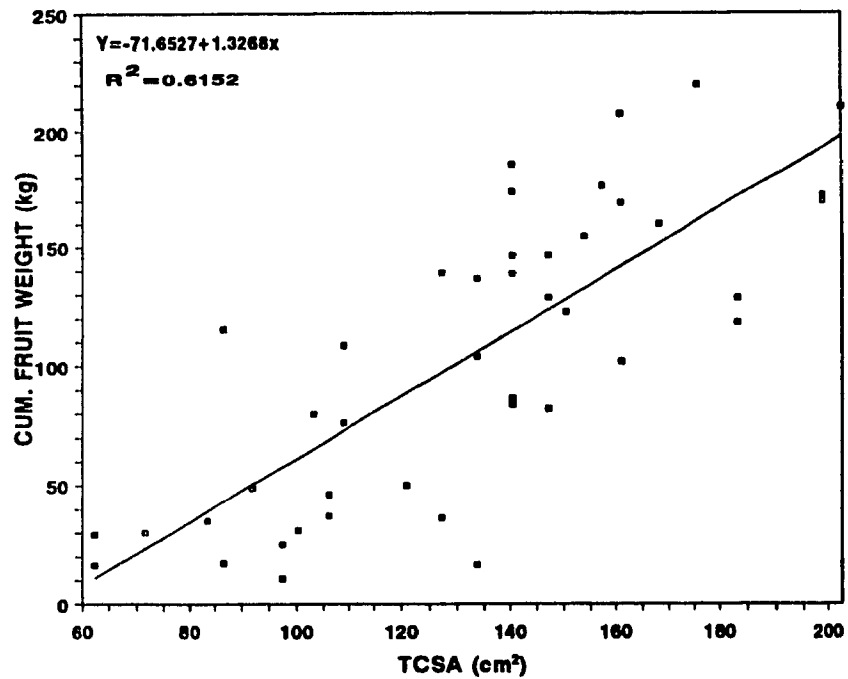


Figure 4.4
Relationship between TCSA 1992 and cumulative yield (fruit weight) 1989-91 of 'Valencia Late' trees.

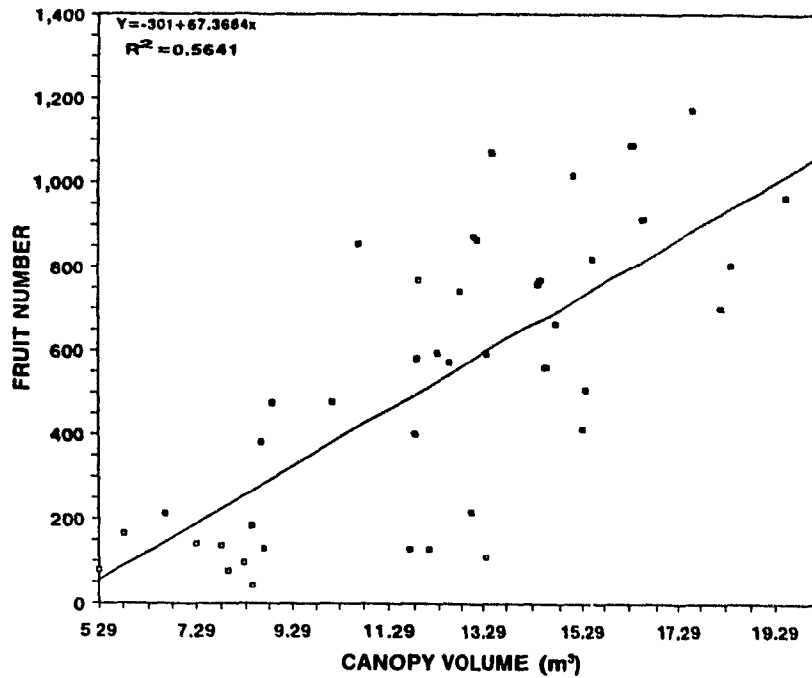


Figure 4.5
Relationship between canopy volume 1992 and cumulative yield (fruit number) 1989-91 of 'Valencia Late' trees.

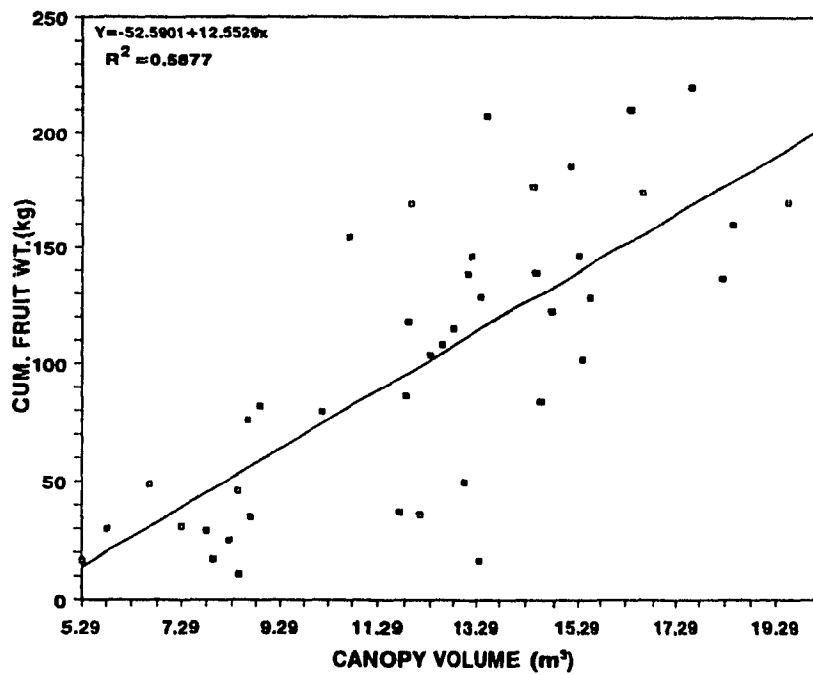


Figure 4.6
Relationship between canopy volume 1992 and cumulative yield (fruit weight) 1989-91 of 'Valencia Late' trees.

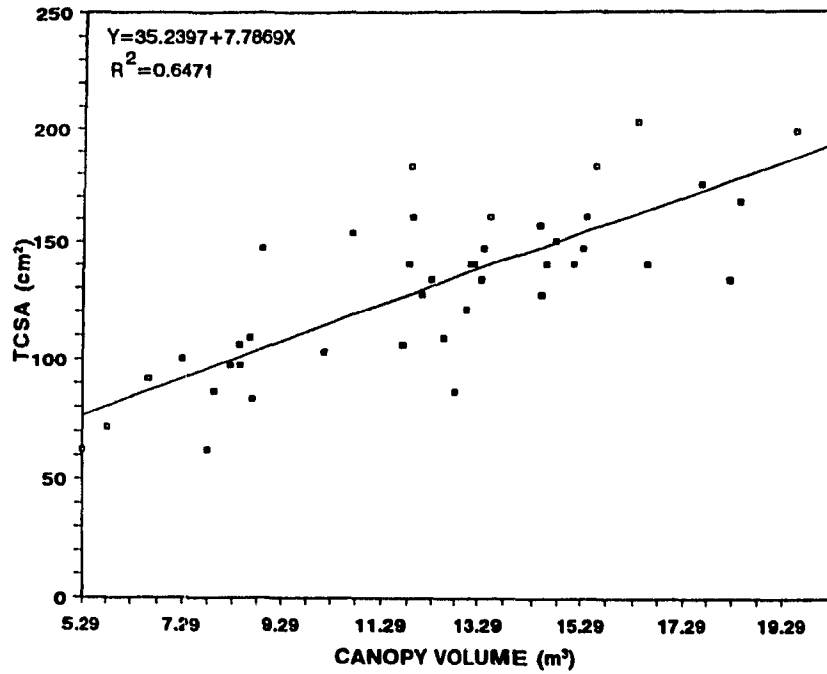


Figure 4.7
Relationship between TCSA and canopy volume of 'Valencia Late' trees in 1992.

TSS was greatest for VL35 and least for VL248 (Table 4.2) 11.09% and 7.10%, respectively. TA was lowest for VL53 and greatest for VL185 (Table 4.2). The TSS/TA ratio varied from 6.47 for VL185 to 19.11 for VL35. Vitamin C content for 'Valencia Late' clones varied from 24.47 mg/100 ml of juice for VL35 to 60.86 mg/100 ml for VL18.

The range in fruit diameter, which determines commercial size classification in fresh fruit, was small, from 7.30 cm for VL18 to 8.23 cm for VL35. (Table 4.2). Minor differences were noticed in the diameter/length ratio with fruits from most of the clones having a value of near 1.0 indicating essentially round fruits. This ratio varied from 1.03 for VL247 to 1.25 for VL53. Peel thickness for these clones varied from 3.25 mm for VL104 to 5.50 mm for VL247 (Table 4.2).

The average fruit weight ranged from 215.3 grams for VL104 to 269.5 grams for VL18 (Table 4.2). 'Valencia Late' fruit was retained on the 'Valencia Late' trees for a long period without loss of quality or fruit abscission.

The preference / acceptance test scores for 'Valencia Late' clones showed that preference was not necessarily directly related to high yield and good growth characteristics in the 'Valencia Late' clones (Table 4.3). In the general appearance test scores VL105 and VL248 had among the greatest scores while VL106 having the lowest. Taste scores showed that VL247 and VL105 had the greatest test scores with VL35 having the lowest. In the texture scores VL18 and VL248 had the

TABLE 4.2 Fruit characteristics of 'Valencia Late' clones 1991

Clone	TSS %	TA %	TSS/TA	Vitamin C mg/100 ml	Length (cm)	Diam (cm)	Diam / length	Peel thickness (mm)	Avg Fruit wt (g)
VL18	8.29	1.15	7.20	60.86	6.73	7.30	1.08	3.70	269.50
VL35	11.09	0.58	19.11	24.47	7.40	8.23	1.11	3.67	256.57
VL53	8.81	0.55	16.02	48.48	6.30	7.90	1.25	4.00	235.74
VL104	8.49	1.12	7.58	41.30	7.05	7.75	1.10	3.25	215.30
VL105	8.49	0.87	9.82	43.48	7.40	8.10	1.09	3.50	256.30
VL106	7.30	0.69	10.55	44.57	7.15	8.10	1.13	3.75	248.30
VL107	7.83	0.93	8.41	34.57	7.03	7.57	1.08	4.67	230.10
VL139	9.40	1.12	8.39	30.85	6.93	7.77	1.12	4.00	222.40
VL185	8.80	1.36	6.47	54.35	7.10	7.45	1.05	3.50	240.00
VL247	9.14	1.06	8.69	56.52	7.20	7.40	1.03	5.50	232.00
VL248	7.10	0.98	7.24	32.98	7.13	7.73	1.08	3.67	232.83

highest scores and VL35 and VL247 the lowest. Acceptability scores showed that VL248 and VL105 were the highest ranking while VL106, VL247 and VL35 were the lowest. The ranking based on the four parameters showed that VL248, VL105 and VL18 were the clones with the greatest mean scores while VL107, VL106 and VL35 were the least.

Table 4.3 Preference / acceptance test scores for 'Valencia Late' fruits' in 1991.

Clone	General appearance	Taste	Texture	acceptability	Mean score
VL18	7.0	6.5	6.8	6.0	6.6
VL35	5.8	3.0	3.2	3.8	4.0
VL104	7.0	5.7	5.7	6.4	6.2
VL105	7.3	6.8	6.2	6.7	6.7
VL106	5.0	4.8	4.5	4.5	4.7
VL107	5.8	5.2	5.6	5.0	5.4
VL139	5.8	5.6	5.7	5.6	5.7
VL185	6.6	5.4	5.3	5.7	5.7
VL247	6.4	7.0	4.4	4.4	5.6
VL248	7.2	6.4	6.6	7.4	6.9

²Scores are the average of 10 tasters on a scale where 1 = very poor and 10 =very good.

4.1.2 'Washington Navel' clones.

4.1.2.1 Growth parameters:

There were differences in trunk cross-sectional area between the 'Washington Navel' clones (Table 4.4), though differences among the clones were not great. WN203 had the greatest TCSA and WN135 the smallest.

Plant height varied among the clones with WN203 having the tallest plants and WN204 the shortest (Figure 4.8). Significant differences were also found in plant canopy width, WN203 was the widest and WN215 was the smallest (Figure 4.9).

There were differences in tree canopy volume (Table 4.4) largest being 21.69 for WN203 to the smallest of 10.09 for WN204.

'Washington Navel' yields were determined in 1990 and 1991 by counting and weighing the fruits at harvest. Clones differed in terms of fruit number and weight (Table 4.4). WN204 had the greatest yield while WN135 had the least when expressed as fruit number. A similar pattern was shown in terms of fruit weight with WN204 producing the greatest weight of fruits and WN135 the least.

Yield efficiency of clones was calculated in terms of fruit number and fruit weight. In either case WN204 was the most productive clone and WN135 the least (Table 4.4).

Table 4.4 Trunk cross-sectional area (TCSA), canopy volume, cumulative fruit yield and yield efficiency of 'Washington Navel' clones.

Clone	TCSA (cm ²)	canopy volume (m ³)	cumulative yield (fruits/tree)	cumulative yield (kg/tree)	cumulative yield efficiency ^x	cumulative yield efficiency ^y
WN39	131.02 bc ^z	15.68 ab	47.75 c	13.55 cde	2.87 d	0.81 c
WN102	115.50 bc	12.84 b	166.50 ab	42.03 abc	12.96 bc	3.26 b
WN135	96.41 c	10.82 b	21.00 c	7.02 e	1.80 d	0.60 c
WN141	139.43 b	10.49 b	82.00 bc	38.00 a-d	7.67 bcd	2.09 bc
WN203	175.96 a	21.69 a	113.50 bc	34.75 a-e	6.30 cd	1.79 bc
WN204	102.68 bc	10.09 b	225.75 a	56.40 a	22.35 a	5.57 a
WN205	101.80 bc	10.86 b	47.00 c	12.53 de	3.99 d	1.07 c
WN215	125.62 bc	11.25 b	180.67 ab	42.73 ab	14.02 b	3.28 b
WN216	124.69 bc	16.68 ab	83.67 bc	19.13 b-e	5.54 d	1.23 c
MEAN	123.19	13.28	107.88	30.01	8.68	2.22
C.V	18.61	29.59	53.90	56.18	50.93	50.25

^x cumulative yield efficiency = cumulative fruit yield (fruit number) 1990-91 / canopy volume 1992.

^y cumulative yield efficiency = cumulative fruit yield (kg) 1990-91 / canopy volume 1992.

^zMeans within a column followed by the same letter are not significantly different from each other at P=0.05 (Duncan's multiple range test).

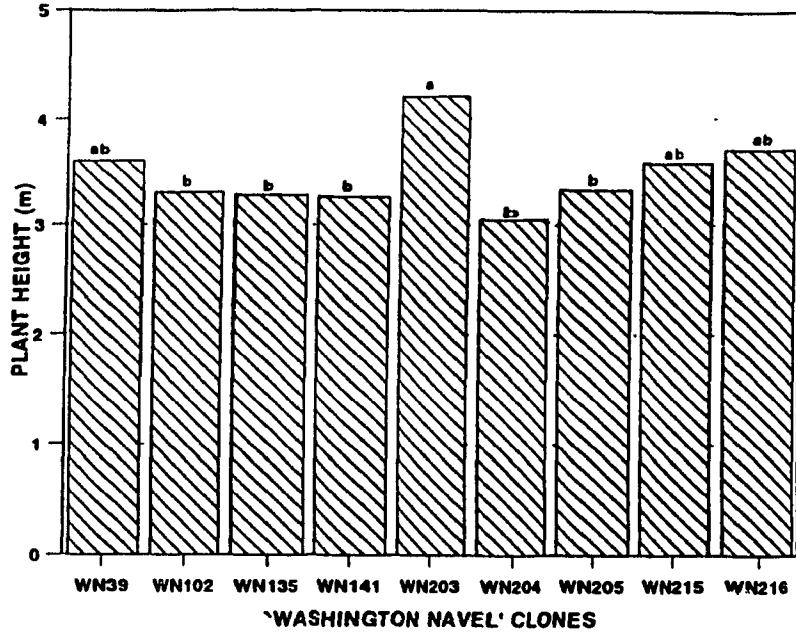


Figure 4.8
Mean plant height of 'Washington Navel' clones (1992).
Bars followed by the same letter are not significantly different by
DMRT at 5% level

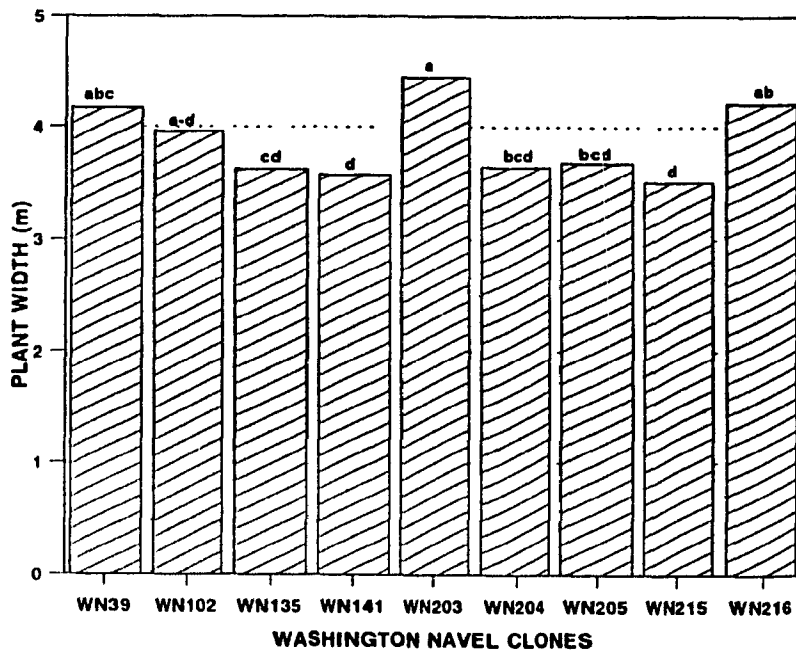


Figure 4.9
Mean plant width of 'Washington Navel' clones (1992).
Bars followed by the same letter are not significantly different by
DMRT at 5% level

The correlation coefficient between TCSA and cumulative fruit yield expressed as fruit number or fruit weight was found to be non-significant being respectively $r= 0.21$, $P=0.24$ and $r= 0.30$, $P=0.096$. Relationship between canopy volume and cumulative fruit yield both in terms of fruit number and weight were both not significant with respectively $r= -0.04$, $P=0.81$ and $r=0.09$, $P=0.63$.

The relationship between canopy volume to TCSA was positively correlated , $r=0.76$, $P=0.01$ (Figure 4.10).

4.1.2.2 Fruit quality: (Table 4.5)

TSS was greatest for WN216 and least for WN135. TA was least for WN135 and greatest for WN216. The TSS/TA ratios showed a variation with WN204 having the greatest and WN216 the least. There was not much variation in vitamin C content but WN39 had the greatest and WN205 the least.

Fruit diameter was fairly uniform and only ranged from 7.50 cm for WN216 to 8.70 cm for WN203. Diameter/length ratio for most of the clones was generally close to one indicating that the fruit shape of most of the clones except WN39 was nearly round. WN39 had the greatest ratio of 1.42 and WN215 had the least of 1.03.

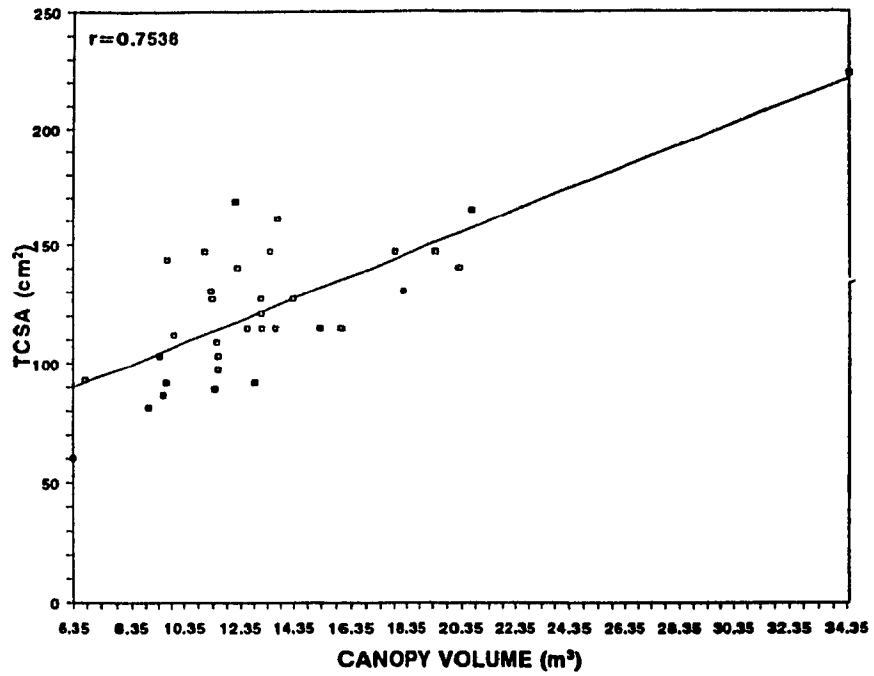


Figure 4.10
 Relationship between TCSA and canopy volume of 'Washington Navel' trees in 1992.

TABLE 4.5 Fruit characteristics of 'Washington Navel' clones 1991

Clone	TSS %	TA %	TSS/TA	Vitamin C mg/100 ml	Length (cm)	Diam (cm)	Diam / length	Peel thickness (mm)	Avg Fruit wt (g)
WN39	10.12	0.82	12.34	64.85	5.50	7.80	1.42	4.00	238.66
WN102	10.44	0.66	15.82	51.52	6.70	7.60	1.13	4.30	219.52
WN135	6.17	0.42	14.83	51.09	7.50	8.10	1.08	6.00	237.60
WN141	10.12	0.88	11.50	58.79	7.90	8.30	1.05	4.00	278.06
WN203	8.81	0.64	13.77	52.72	7.30	8.70	1.19	4.30	320.00
WN204	10.44	0.49	21.31	63.03	7.40	8.00	1.08	4.30	238.06
WN205	9.47	0.51	18.57	50.30	7.20	8.20	1.14	4.30	281.20
WN215	9.79	0.81	12.09	52.73	7.70	7.90	1.03	4.00	270.28
WN216	10.76	1.04	10.35	54.55	6.80	7.50	1.10	4.00	208.76

The peel thickness ranged from a minimum of 4.0 mm for most of the clones to a maximum of 6.0 mm for WN135. Fruit weight varied from 208.76 grams for WN216 to 281.20 grams for WN205.

Granulation, a physiological disorder was generally observed to be more pervasive and severe in 'Navel' clones than 'Valencia Late' clones stored on the tree after reaching maturity and resulted in quality loss.

Preference / acceptance test scores showed that most of the clones that had high cumulative fruit yield and also high cumulative yield efficiency were also among those high ranking in the parameters considered (Table 4.6). In the general appearance scores WN216 and WN102 were ranking highest with WN135 being the lowest. Taste scores showed that WN102 and WN39 were the best with WN205 and WN135 being the worst. The texture scores showed that WN39, WN215 and WN216 had among the greatest scores with WN205 and WN135 having the least. In the general acceptability test WN203, WN204 and WN102 had among the highest scores with WN205 and WN135 being the lowest scoring clones. When the mean score for the four parameters were taken into consideration, WN102, WN39, WN216 and WN204 were found to be among the highest ranking while WN205 and WN135 were lowest ranking.

Table 4.6 Preference / acceptance test scores for 'Washington Navel' fruits' in 1991.

Clone	General appearance	Taste	Texture	acceptability	Mean score
WN39	7.0	7.4	7.0	7.1	7.1
WN102	7.2	7.5	6.4	7.2	7.1
WN135	3.5	4.3	3.9	3.9	3.9
WN141	6.2	5.5	5.4	6.4	5.9
WN203	6.5	6.7	5.5	7.3	6.5
WN204	7.0	7.0	5.7	7.3	6.8
WN205	5.5	4.5	4.7	4.9	4.9
WN215	6.2	7.0	6.5	7.0	6.7
WN216	7.3	7.2	6.5	7.1	7.0

²Scores are the average of 10 tasters on a scale where 1 = very poor and 10 =very good.

4.2 Postharvest degreening of citrus fruits.

4.2.1 Experiment 1: Determination of the influence of ethephon rates and number of dips on fruit colour and postharvest decay.

Analysis of variance showed that there were significant differences among treatments where different ethephon concentrations were used (Table 4.7). Ethephon at 2000 ppm induced the numerically highest colour reading using the colour chart i.e., 8.525 though this was not significantly different from that induced by the rates of 2500 and 3000 ppm (Figure 4.11). The control at zero ppm ethephon resulted in the lowest colour score of 6.150.

There was no significant effect of dipping once or twice and the interaction between number of ethephon dips and ethephon rate was non-significant in the model.

4.2.2 Experiment 2: Determination of ideal ethephon rate for postharvest degreening.

4.2.2.1 Experiment 2 (June 11, 1992):

Analysis of variance showed that significant differences in colour were found five days after treatment with the control rate of zero ppm ethephon differing from ethephon treated fruits (Table 4.8 and Figure 4.12). The colour score increased progressively during storage, though no significant differences were found among the ethephon treated fruits until 18 days after treatment. Greatest noticeable colour change among the ethephon treated fruits was induced in the first

Table 4.7 Influence of ethephon rates^x and the number of dips on fruit colour (Experiment 1).

Ethephon ppm	colour
0	6.150 d
500	7.450 c
1000	7.925 b
1500	8.163 ab
2000	8.525 a
2500	8.262 ab
3000	8.237 ab

Mean separation by DMRT at 5% level

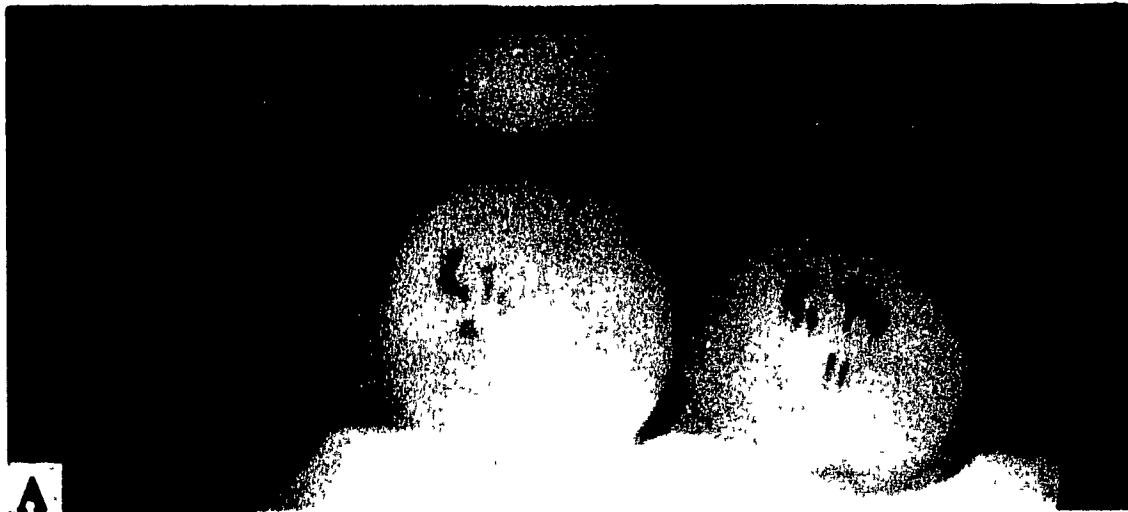
Means with the same letter(s) are not significantly different.

^x This was 15 days after treatment.

Table 4.8 Effect of ethephon rates on post harvest degreening at 0, 5, 11, and 18 days after treatment. Experiment 2 (June 11, 1992).

Ethephon treatments (ppm)	Rind colour rating ²			
	Storage period in days			
	0	5	11	18
0	5.30	5.35 b	5.58 b	5.80 c
500	5.30	6.78 a	7.20 a	7.28 b
1000	5.30	7.10 a	7.78 a	7.90 ab
1500	5.25	6.78 a	7.05 a	7.75 ab
2000	5.30	7.03 a	7.88 a	8.38 ab
2500	5.25	6.90 a	7.90 a	8.08 ab
3000	4.98	6.80 a	7.90 a	8.60 a
Mean	5.24	6.68	7.33	7.68
C.V	3.60	8.36	8.30	10.08

²Means within a column followed by the same letter are not significantly different from each other at p=0.05 (Duncan's multiple range test).

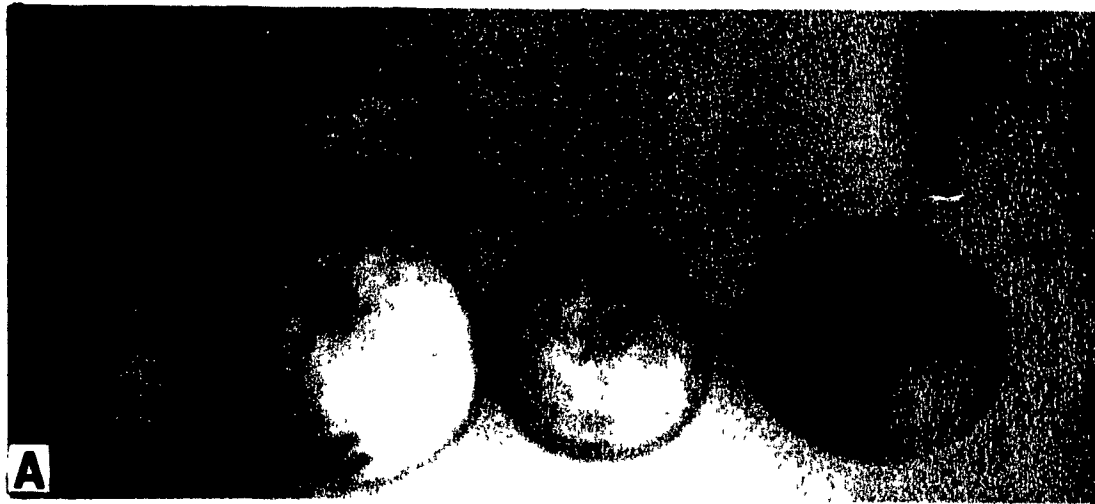


A
0 ppm 2500 ppm 3000 ppm
Fruits which had a second dip in ethephon.



B PEST ATTACK

Figure 4.11 Fruit response to ethephon treatment, (A) at different concentrations; (B) colour arrest due to preharvest pest attack.



1500 ppm

1000 ppm

500 ppm

0 ppm



2500 ppm

0 ppm

2500 ppm

Figure 4.12 (A) and (B) Fruit response to ethephon treatment at different concentrations five days after treatment.

five days and this diminished with increasing period of storage. Eleven days after treatment, fruits exposed to 2000 - 3000 ppm ethephon had more yellow colour than treatments with lower ethephon rates and control rate of zero ppm ethephon.

4.2.2.2 Experiment 2 (July 14, 1992):

Analysis of variance showed that there were significant differences among the ethephon treatments six days after treatment (Table 4.9). At this time the zero and 500 ppm ethephon treatments had the lowest colour scores and these were significantly different from the rest of the ethephon treatments. Eleven days of storage resulted in induction of more yellow colour in fruits which had received 2000 to 3000 ppm ethephon. After storage for 22 days, a more yellow colour was induced in fruits treated with ethephon rates greater than 500 ppm but no significant differences were found among these treatments. Significant differences were found between zero and 500 ppm ethephon treatments and between them and treatments greater than 500 ppm.

4.2.2.3 Experiment 2 (July 28, 1992).

Analysis of variance showed that significant differences in colour induction were evident seven days after treatment and that ethephon rates of 500 ppm and higher differed from the control (Table 4.10). At 13 days after treatment greater yellow colour induction could be noticed in treatments with ethephon rates greater than 1500 ppm. The greatest colour induction among the ethephon treated fruits was noticed during

the first week after treatment.

Table 4.9 Effect of ethephon rates on postharvest degreening at 0, 6, 11 and 22 days after treatment. Experiment 2 (July 14, 1992).

Ethephon treatments (ppm)	Rind colour rating ²			
	Storage period in days			
	0	6	11	22
0	5.18	5.10 c	5.38 d	5.78 c
500	5.10	6.08 b	6.85 c	7.08 b
1000	5.23	7.28 a	7.75 ab	8.10 a
1500	5.10	6.88 a	7.43 b	7.98 a
2000	5.15	7.03 a	7.95 a	8.35 a
2500	5.15	7.28 a	7.95 a	8.10 a
3000	5.20	7.18 a	7.88 ab	8.43 a
Mean	5.16	6.69	7.31	7.69
C.V	1.83	5.21	4.06	5.67

²Means within a column followed by the same letter are not significantly different from each other at p=0.05 (Duncan's multiple range test).

Table 4.10 Effect of ethephon rates on postharvest degreening at 0, 7 and 13 days after treatment. Experiment 2 (July 28, 1992).

Ethephon treatments (ppm)	Rind colour rating ²		
	Storage period in days		
	0	7	13
0	5.05	5.50 b	6.13 d
500	5.08	6.48 a	7.23 c
1000	4.98	6.55 a	7.33 bc
1500	5.03	6.93 a	7.98 ab
2000	5.10	7.40 a	8.03 ab
2500	5.10	7.38 a	8.18 a
3000	5.10	6.83 a	8.03 ab
Mean	5.06	6.72	7.55
C.V	1.78	8.30	6.16

²Means within a column followed by the same letter are not significantly different from each other at p=0.05 (Duncan's multiple range test).

4.2.3 Determination of the influence of light on fruit response to ethephon.

4.2.3.1 Experiment 3 (July 14, 1992): Determination of the influence of light on fruit response to ethephon.

Analysis of variance showed a high significant effect of fruit response to ethephon treatments at 0.1% level of significance. Fruits that were dipped in ethephon and kept in the light showed a progressive colour change with increasing ethephon concentrations but there was no similar relationship in colour induction for those kept in the dark (Table 4.11). For fruits treated and kept in the light, control treatment had the lowest colour change and treatment with 3000 ppm the greatest.

There were no significant differences between the simple effects of light or no light treatments but an interaction existed between ethephon rates and light treatments that was significant at a level of 0.1%. (Table 4.11).

Table 4.11 Influence of light on fruit response to ethephon. Experiment 3 (July 14, 1992).

Ethephon ppm	no light	light
0	7.85	5.48
500	7.45	6.73
1000	6.85	7.75
1500	7.70	7.68
2000	7.65	8.13
2500	7.70	7.93
3000	7.53	8.20

Means of the interaction between the ethephon rates and influence of light treatments.

4.2.3.2 Experiment 3 (July 28, 1992): Determination the influence of light regime on fruit response to ethephon.

Analysis of variance showed that there was a significant effect of ethephon treatments on fruit colour change. This was significant at 0.1% with induction of colour progressively increasing with higher ethephon concentrations. The control treatment at zero ppm ethephon had the lowest colour score of 6.250 while ethephon rate at 2500 ppm had the greatest of 8.075 that was not significantly different from that of ethephon rate at 2000 ppm (Table 4.12).

There was also a significant effect at 0.1% level of the influence of light regime on fruit colour change with fruits treated and kept in light or no light having respective mean colour scores of 7.821 and 7.207. These means were significantly different at 5% according to the DMRT. There was a non-significant interaction between the ethephon rates and

light or no light treatments.

Results of the study showed that in all these experiments there was a non-significant difference in fruit rot due to the ethephon treatment. In experiment 3 carried out on July 28, 1992 fruit rot incidence was found to be influenced by light regime and this was significant at 0.1% with the rot being higher in the dark compared to that in the light. The means were respectively 2.04 and 0.42 which were significantly different at 5% according to the Duncan's multiple range test.

Table 4.12 Influence of light on fruit response to ethephon. Experiment 3 (July 28, 1992).

Ethephon ppm	colour
0	6.250 c
500	7.175 b
1000	7.625 ab
1500	7.812 a
2000	7.850 a
2500	8.075 a
3000	7.812 a

Mean separation by DMRT at 5% level

Means with the same letter(s) are not significantly different.

5 DISCUSSION

5.1 Citrus clone evaluation:

The advantages to the grower of one 'Valencia Late' or 'Washington Navel' selection over another must be evaluated considering factors of production in the early years, consistency during the expected life of the tree, total production and canopy efficiency, to provide adequate yields while limiting tree size. Thorough evaluation of the genetic characters of new varieties requires they be observed in various environments over a long period of time with meaningful yield and fruit quality data being based on more than one harvest. In this study the clones were planted in a citrus greening disease-free site and growth and fruiting characteristics were evaluated to determine the best clones for use in national performance trials. Some differences between clones were noticed in the growth and fruiting characteristics but they were still in their early years of growth, a period when a lot of variations are expected. Broadbent (1987) recommended several years of observations of trees and fruits for cropping and genetic abnormalities (chimeras) before budwood is supplied from mother-block trees. Results of a study on 'Valencia Late' (Rouse and Maxwell 1988) showed that data collected over a number of years should be used when selecting clones for an area. Evaluation of twenty-years old trees over a sixteen year period of production showed that the first six years represented only 10% of the

cumulative total production. Years seven to eleven accounted for 30% of the cumulative total, with 50% of the trees' production being realised in the last four years.

High yield is not always synonymous with optimum growth. Although the largest trees are often the heaviest producers, tree size is not a good indicator of productivity of trees (Colburn et al. 1963). Significant difference in tree size present among both 'Valencia Late' and 'Washington Navel' clones was in general agreement with Marloth et al. (1964) who reported significant differences in tree size among strains of 'Valencia' orange on rough lemon rootstock in his 15 year study. 'Washington Navel' clones were relatively slow in coming to bearing when compared with the 'Valencia Late' clones.

There was a strong correlation between fruits in terms of number or weight and TCSA for the 'Valencia Late'. A positive correlation indicates a high degree of predictability in the use of the parameter to estimate tree bearing capability. A positive correlation between canopy volume and fruits in terms of numbers or weight was in general agreement with Wheaton et al. (1991).

Greatest cumulative yield efficiencies (kg) were observed in 'Valencia Late' clones such as VL106, VL139 and VL185 which also had among the largest tree sizes. However 'Washington Navel' clone 204 had the smallest canopy volume and the greatest cumulative yield efficiency (kg) with WN216

and WN39 having the least; though their canopy volumes were among the greatest. Young *et al.* (1968) reported that low cumulative yield of trees could be caused by the fact that nucellar trees are known to come to bearing late and are often poor producers as young trees; this was noticed in some 'Valencia Late' clones e.g, VL18 and 'Washington Navel' clones. The low cumulative yields resulted in lowered cumulative yield efficiencies. A combination of a clone with a good yield/volume ratio is desirable for high density planting (Ashkenazi and Oren 1977). Roose *et al.* (1989) reported that a combination of high yield efficiency and smaller tree size may be most suitable for high density planting.

The variations observed in yield among 'Valencia' and 'Washington Navel' clones could be caused by juvenility due to the use of seedlings as rootstocks. According to Cary *et al.* (1977) such rootstocks cause pronounced juvenile characteristics in the scion, such as excessive vegetative growth and a reluctance to flower and set fruit which can persist for the first five to six years.

There was a significant positive correlation between the trunk cross-sectional area and canopy volume in both 'Valencia' and 'Washington Navel' clones. This is in agreement with work on apples reported by Westwood and Robert (1970) who found that TCSA was highly correlated to canopy volume; although according to Roose *et al.* (1989) this correlation in

citrus is highly influenced by the rootstock genotype. Wheaton et al. (1991) reported $r^2 = 0.79$, $P < 0.01$ between trunk diameter and canopy volume. Fallahi et al. (1990) however reported contrasting results on lemon cultivars where they found that TCSA was not proportional to canopy volume in all cultivars. In this study the simple correlation between TCSA and canopy volume data provided some logical and fairly consistent relationships. This shows the possibility of either of the two being used in the estimation of yield efficiency.

Fruit juice composition can vary from year to year reflecting seasonal climatic variations. Fruits with a TSS/TA ratio of 7.5:1 to 9:1 are considered mature depending on the standards of the growing area (Davies 1986). In this study the ratio was found to be generally greater than eight among 'Washington Navel' clones and such fruits would even be considered mature by the United States standards. Fruit quality data for 'Washington Navel' clones showed that the juice TSS/TA ratio reached was satisfactory for the fresh market. For the 'Valencia Late' clones fruit, TSS/TA ratio greater than eight would readily be acceptable by commercial fruit processing factories; and of the highest yielding three clones, only VL106 and VL139 had TSS/TA ratios greater than eight. 'Valencia Late' clones had generally lower TSS/TA ratios than 'Washington Navel' clones. TSS are reported to be lower in fruits from young trees but to increase notably as trees age (Cameron and Frost 1968) and so their levels would

be expected to change as the trees in the mother-block age.

Vitamin C content also showed some variation between varieties with 'Washington Navel' clones generally having higher levels than 'Valencia Late' clones.

The fruit shape, peel thickness and average size may be influenced by the climate. Data on fruit diameter/length show that fruit diameter increased more rapidly than length in both 'Valencia Late' and 'Washington Navel' clones; if these findings are valid then this would contradict the findings reported on 'Valencia Late' by Jahn (1970). A ratio of one indicates that the fruit is essentially round. Lower values indicate a more elongated shape while higher values indicate a flatter shape according to Jahn (1970).

Peel thickness may be strongly influenced by temperature during the second and third stages of fruit development. Stage one in citrus fruit development is the period of cell division and includes flowering and formation of various tissues in the small fruits. Stage two follows the cell division and is characterised by enlargement of the cells in the fruit. In the later part of stage two peel changes colour from green to orange in sub-tropical and Mediterranean regions. Stage three is the maturation period and is characterized by reduced rate of enlargement (Bain 1958). The greatest peel thickness for 'Valencia Late' clones was found to be 5.5 mm and this is within an acceptable range. Peel thickness of 'Washington Navel' fruits was also within an acceptable range for

'Washington Navels' on rough lemon rootstock. Levy and Mendel (1982) working on 'Washington Navel' reported a peel thickness of 5.8 mm. Peel thickness can vary greatly from year to year (Fallahi et al. (1989) and is only an important consideration if fruits are for the fresh market, it is not a problem for processing varieties.

Average fruit weight among the 'Valencia Late' and 'Washington Navel' clones was close to uniform in these experiments and did not seem to correlate with yield. Cumulative yield data for 'Valencia Late' and 'Washington Navel' were for the years 1989-91 and 1990-91 respectively and average fruit weight was based only on the 1991 yield data. This could explain why the effect of crop load on the fruit size was not found to be significant. To a great extent the average size of the fruit produced in any tree of a given cultivar is related to the size of the crop, fruit generally being smaller on trees bearing heavier crops (Marloth et al. 1964, Wheaton et al. 1991).

In citrus organoleptic tests, taste relates best to TSS or TSS/TA ratio. For mature fruits a tarter fruit i.e., one with a higher acid content may be more acceptable than a sweeter one if the former also has a higher °Brix. This is reported to occur due to the buffering action of potassium and other salts in the juice (Soule and Grierson 1986b). One would have expected better scores from fruits with a high TSS/TA ratio but the results suggest that flavour is more

complicated than a simple relation of TSS/TA ratio (Onayemi 1977). This could be a possible reason to explain the lack of consistency between the organoleptic scores and the TSS or TSS/TA ratios in the tested clones.

In the fruit preference / acceptance test scores, results showed that unlike in 'Valencia Late' clones, a high consistency was found in 'Washington Navel' clones in that clones with greatest scores in cumulative yield and cumulative yield efficiency had generally greatest preference / acceptance scores. 'Washington Navel' is generally used for the fresh fruit market while 'Valencia Late' is for juice processing. The overall pattern of fruit preference / acceptance followed no pattern that could be discerned and was as reported in work by Onayemi (1977). Except for VL35 and WN135 which had respective mean scores of 4.0 and 3.9 and could therefore be said to be poor, the rest of the clones could all be classified as fair.

5.2 Postharvest degreening of citrus fruits:

The colour development trend exhibited indicated that with every increase in the concentration of ethephon there was an appreciable degradation of chlorophyll and production of brighter yellow fruits over control. Similar effects of accelerated destruction of chlorophyll due to ethrel application in many citrus species have been reported by Fuchs and Cohen (1969), Arora *et al.* (1973), and Josan *et al.* (1981).

Fruits packed in aluminium foil were glossier compared to those dipped in ethephon and kept in light. This was due to the minimum shrivelling of wrapped fruits observed with increased storage compared to those in open boxes. The aluminium foil prevented the evaporation of moisture from fruits resulting in better appearance after storage.

A complete orange coloration like that found in areas where temperatures are favourable for natural degreening on the tree was not achieved. Fruits treated with ethephon showed better colour development that was noticeable by the third day, and with the greatest increase in colour intensity being observed up to seven days after treatment, thereafter very little colour change occurred. There was appreciable degradation of chlorophyll resulting in more yellow pigmentation of rind in these experiments but no development of typical orange colour and was as reported by Arora *et al.*

(1973). Although treated fruits did not attain the natural and desired colour, they were more attractive than untreated ones. According to Long (1964) and Akamine et al. (1975) fruits may still remain yellow or very pale orange in colour after degreening and dyeing (colour add) can be used to intensify the colour for greater consumer appeal. Citrus red number 2 which is 1-(2,5-dimethoxyphenylazo)-2-naphthol with an established tolerance of 2 ppm is of general use in the colour adding.

Pest attack was found to inhibit coloration in the post-harvest degreening experiments using ethephon (Figure 4.11). Similar observations have been reported with ethylene use by Grierson and Newhall (1960).

Dipping fruits a second time three days after the first dip did not induce any significant change in fruit colour. Young and Otto (1972b) had reported that a preharvest double application of 200-500 ppm ethephon on 'Robinson' tangerine significantly reduced chlorophyll levels over single sprayed trees. In their study this double spray also resulted in more fruit drop than single sprays.

The effect of light showed contrasting results. In experiment 3 (July 14, 1992) there was no effect while in experiment 3 carried out on July 28, 1992 light did influence response. These differences in response could have been attributed to differences in the storage methods after treatment. In experiment 3 of July 14, 1992, fruits were

stored in dark compartments after treatment while in experiment 3 of July 28, 1992 they were kept in open boxes, though wrapped in aluminium foil. Such differences in fruit response have been reported by other workers. Arora *et al.* 1973 on 'Valencia', and Rana and Chauhan 1976 on "Kagzi lime", reported there being no remarkable difference in colour development in ethephon treated fruits when kept in light or dark; but fruits in the dark looked more fresh than others, we observed the same phenomenon in our studies. Obervacher (1968) and El-Zeftawi *et al.* (1978) had reported that degreening was somewhat slower in the dark than in the light.

Though Minessey *et al.* (1972) reported the rate of degreening of grapefruits as function of both the 'ethrel' concentration and the length of storage period, our study showed that the highest colour change occurred within the first week after treatment. Subsequent colour change were less and nearly negligible since longer storage also resulted in fruit shrivelling and therefore quality loss.

There was no significant differences in decay among the ethephon treatments used. Results were in general agreement with those of Hatton and Cubbege (1973) on grapefruit, El-Hammady *et al.* (1974) and Young *et al.* (1974) who reported that ethephon-sprayed fruits decayed at rates equal to or less than those of unsprayed fruit. But they contrasted those of other workers (Grierson and Newhall 1960, McCornack 1971, Jahr 1973, Cohen 1978, Brown 1986) who found out that postharvest

degreening of citrus fruits with ethylene is frequently accompanied with high incidence of decay. Cultivar differences, suitable concentrations, and time and method of application are probably responsible for varied responses. In our study differences might also have been caused by the much smaller sample size used, which was insufficient to detect any consistent and significant treatment effect.

6 CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY.

6.1 Citrus clone evaluation:

The purpose of this study was to evaluate the introduced clones and determine the best for use in the national performance trials. Although it is difficult to select a single clone with all the desirable characteristics, the superior yield and fruit quality of VL106 and VL139 suggest that they would perform well and be good candidates for commercial production. WN204 was the best among the 'Washington Navel' clones based on fruit yield and fruit yield efficiency. VL185 though among the clones with good growth characteristics and high yields had a low TSS/TA ratio; however this characteristic has been reported to improve notably as the tree ages (Cameron and Frost 1968). Also the use of different rootstocks may influence a TSS/TA ratio. Rough lemon, a vigorous rootstock that is the standard rootstock for this area is reported to impart low TSS in the fruits (Davies 1986). Fruit internal characteristics i.e., level of aromatic constituents, TSS, TA, TSS:TA ratio, fruit age and juice content all influence palatability. These vary from one year to another, and there are substantial variations during the crop season. Therefore the most important factor of all in any selection, is the likes and dislike of the individual who eats the fresh fruit or drinks its juice.

'Valencia Late' was found to have an ability to hold or

store fruit on the tree long which is considered to be a useful field characteristic. Granulation, a physiological disorder was generally more pervasive and severe in Navel clones than 'Valencia' stored on the tree after reaching maturity as reported by Davies (1986). This therefore necessitates the prompt picking of mature fruits from the 'Washington Navel' clones.

Risks are of course unavoidable in the adoption of any new varieties, and final conclusions will emerge only after extended trials and experience under a wide range of growing conditions. It is therefore essential that the search for better clones be continued and a true evaluation of the all-round suitability of the final choice be based on a series of long term comparative tests. In future evaluations of clones and selections it is vital that plantations be laid out in such a way as to allow for analysis of yield and growth data from the trees. Such evaluation should include work on the effect of different rootstocks on the yield, fruit quality and disease resistance in the citrus growing areas. Results from such trials will aid us in the identification of clones and also rootstocks suitable for different citrus growing areas in Kenya.

6.2 Postharvest degreening of citrus fruits:

Studies should be continued in an effort to improve the art of degreening citrus fruits on a small scale farm as rapidly as possible. These colour changes should be obtained

as rapidly as possible. After harvest fruits constantly give off water vapour, techniques to control relative humidity through modification of degreening structures and storage facilities will help in maintaining high quality fruits postharvest. Fruits ripen better and have a better appearance with the absence of shrivelling. This rate of loss of water vapour is reported by Grierson and Newhall (1960) to inversely vary with the relative humidity of the degreening room.

The results obtained in these investigations show that 2000 ppm ethephon gave a satisfactory colour for treated fruits and this rate should be included in any future work. Though the results of the study showed a non-significant effect of ethephon treatment on decay in postharvest degreening of citrus fruits further studies need to be done with large sample sizes of over 100 fruits to verify this.

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APPENDICES

Appendix A: Information on the citrus clones:

'Washington Navel' clones:

WN39: 'Washington Navel' U.S.A. S1D13-3: Line introduced in seed form from Tunisia in 1961.

WN102: 'Washington Navel' Rhylen: received in graft form from Boufarik Station Algeria in 1963.

N135: 'Navel' S1D13-12: Line received in seed form from Ariana Station, Tunisia in 1966.

WN141: 'Washington Navel' Parent: Line introduced in graft form from Riverside station California in 1966.

WN203: 'Washington Navel': Line received in graft form from Boufarik Station, Algeria in 1971.

WN204: 'Washington Navel': Line stemming from nucellar selection by P.J.Cassin, San Giuliano Station, (Corsica).

WN205: 'Washington Navel': Line stemming from nucellar selection by P.J.Cassin, San Giuliano Station (Corsica).

WN215 'Washington Navel': Line stemming from nucellar selection by P.J.Cassin, San Giuliano Station (Corsica).

WN216 'Washington Navel': Line stemming from nucellar selection by P.J.Cassin San Giuliano Station (Corsica).

'Valencia Late' clones:

VL18: 'Valencia Late' Olinda: Received in graft form from Riverside Station (California) in 1961.

VL35: 'Valencia Late' Alibert: Line introduced from Verger Alibert in Tunisia in graft form in 1965.

VL53: 'Valencia Late' Floride 10-12-7-X: line introduced in graft form from I.F.A.C. Station, Morocco in 1964.

VL104: 'Valencia Late' Campbell R4: Line received in graft form from Boufarik Station (Algeria) in 1963.

VL105: 'Valencia Late' Campbell R37: Line received in graft form from Boufarik Station (Algeria) in 1963.

VL106: 'Valencia Late' Olinda 2750: Line introduced in

graft form from Boufarik Station (Algeria) in 1963.

VL107 : 'Valencia Late': Line introduced in graft form from Boufarik Station (Algeria) in 1963.

VL139: 'Valencia Late Frost': Line introduced in graft form from Willits and Newcomb Society, Riverside California in 1966, and this material was introduced to Corsica from I.F.A.C. Station Martinica (French West Indies).

V185: 'Valencia Cutter': Line introduced in graft form from Willits and Newcomb Society, Riverside California in 1966.

VL247: 'Valencia Late': Line is from nucellar selection by P.J.Cassin, San Giuliano Station (Corsica).

VL248: 'Valencia Late': Line is from nucellar selection by P.J.Cassin, San Giuliano Station (Corsica).

(Source: C. Chabrier INRA-Corsica, Personal communication 1993).

Appendix B: Analysis of variance

Appendix 1. Analysis of variance for plant height of 'Valencia Late' clones, 1992.

Clone	DF	MS	F	Prob > F
Clones	10	0.132	1.29	0.276
Error	33	0.103		
R-square	0.28			
C.V.	9.27			

Appendix 2. Analysis of variance for tree canopy width of 'Valencia Late' clones, 1992.

Clone	DF	MS	F	Prob > F
Clones	10	0.667	6.50	0.0001
Error	33	0.102		
R-square	0.66			
C.V.	8.55			

Appendix 3. Analysis of variance for trunk cross-sectional area of 'Valencia Late' clones, 1992.

Source	DF	MS	F	Prob > F
Clones	10	34764.90	5.28	0.0001
Error	32	657.9857		
R-square	0.62			
C.V.	19.38			

Appendix 4. Analysis of variance for tree canopy volume of 'Valencia Late' clones, 1992.

Clone	DF	MS	F	Prob > F
Clones	10	36.51	5.21	0.0001
Error	33	7.00		
R-square 0.61				
C.V.	21.25			

Appendix 5. Analysis of variance for cumulative fruit yield (in fruit numbers) during 1990-91 for 'Valencia Late' clones.

Source	DF	MS	F	Prob > F
Clone	10	292231.56	5.07	0.0002
Error	33	57675.52		
R-square 0.61				
C.V.	44.98			

Appendix 6. Analysis of variance for cumulative fruit yield (in kilograms) during 1989-91 for 'Valencia Late' clones.

Source	DF	MS	F	Prob > F
Clone	10	10384.66	6.01	0.0001
Error	33	1726.91		
R-square 0.65				
C.V.	40.28			

Appendix 7. Analysis of variance for cumulative fruit yield efficiency (in fruit number) during 1989-91 for 'Valencia late' clones.

Source	DF	MS	F	Prob > F
Clones	10	971.52	3.81	0.0017
Error	33	254.90		
R-square 0.54				
C.V.	39.83			

Appendix 8. Analysis of variance for cumulative fruit yield efficiency (in kilograms) during 1989-91 for 'Valencia Late' clones.

Source	DF	MS	F	Prob > F
Clones	10	33.36	4.79	0.0003
Error	33	6.96		
R-square 0.59				
C.V.	33.85			

Appendix 9. Analysis of variance for plant height of 'Washington Navel' clones, 1992.

Clone	DF	MS	F	Prob > F
Clones	8	0.463	2.63	0.030
Error	26	0.176		
R-square 0.45				
C.V.	12.10			

Appendix 10. Analysis of variance for tree canopy width of 'Washington Navel' clones, 1992.

Clone	DF	MS	F	Prob > F
Clones	8	0.440	3.64	0.0058
Error	26	0.121		
R-square 0.53				
C.V.	9.02			

Appendix 11. Analysis of variance for trunk cross-sectional area of 'Washington Navel' clones, 1992.

Source	DF	MS	F	Prob > F
Clones	8	2352.60	4.47	0.0018
Error	25	525.82		
R-square 0.59				
C.V.	18.61			

Appendix 12. Analysis of variance for tree canopy volume of 'Washington Navel' clones, 1992.

Clone	DF	MS	F	Prob > F
Clones	8	59.67	3.86	0.0041
Error	26	15.45		
R-square 0.54				
C.V.	29.59			

Appendix 13. Analysis of variance for cumulative fruit yield (in fruit numbers) during 1990-91 for 'Washington Navel' clones.

Source	DF	MS	F	Prob > F
Clone	8	18193.84	5.38	0.0006
Error	24	3380.62		
R-square 0.64				
C.V.	53.90			

Appendix 14. Analysis of variance for cumulative fruit yield (in kilograms) during 1990-91 for 'Washington Navel' clones.

Source	DF	MS	F	Prob > F
Clone	8	1082.78	3.81	0.0051
Error	24	284.16		
R-square 0.56				
C.V.	56.18			

Appendix 15. Analysis of variance for cumulative fruit yield efficiency (in fruit number) during 1990-91 for 'Washington Navel' clones.

Source	DF	MS	F	Prob > F
Clones	8	169.03	8.65	0.0001
Error	24	19.55		
R-square 0.74				
C.V.	50.93			

Appendix 16. Analysis of variance for cumulative fruit yield efficiency (in kilograms) during 1990-91 for 'Washington Navel' clones.

Source	DF	MS	F	Prob > F
Clones	8	9.86	7.93	0.0001
Error	24	1.24		
R-square 0.73				
C.V.	50.25			

Appendix 17. Analysis of variance of the influence of ethephon rates and the number of dips on fruit colour, Experiment 1.

Source	DF	MS	F	Prob > F
Ethephon conc E	6	5.228	28.50	0.0001 ***
Replicates	3	2.017	11.00	0.0001 ***
Number of dips D	1	0.002	0.01	0.9259 **
ED	6	0.383	2.09	0.0770 **
Error	39	0.183		
R-square 0.85				
C.V.	5.48			

*** = significant at 0.1% level, ** = not significant.

Appendix 18. Analysis of variance of the influence of light on fruit response to ethephon. Experiment 3. (July 14 1992).

Source	DF	MS	F	Prob > F
Ethephon conc E	6	1.76	7.07	0.0001 ***
Replicates	3	0.38	1.54	0.2204 ns
Light/no light L	1	0.21	0.83	0.3681 ns
EL	6	2.54	10.18	0.0001 ***
Error	39	0.25		
R-square	0.74			
C.V.	6.68			

*** = Significant at 0.1% level, ns = not significant.

Appendix 19. Analysis of variance of the influence of light on fruit response to ethephon. Experiment 3. (July 28, 1992).

Source	DF	MS	F	Prob > F
Ethephon conc E	6	3.11	12.08	0.0001 ***
Replicates	3	1.45	5.65	0.0026 **
Light/no light L	1	5.28	20.54	0.0001 ***
EL	6	0.11	0.43	0.8517 ns
Error	39	0.26		
R-square	0.74			
C.V.	6.75			

*** = Significant at 0.1% level, ns = not significant.

Appendix 20. Analysis of variance of the influence of light on fruit rot in ethephon treated fruits. Experiment 3. (July 28, 1992).

Source	DF	MS	F	Prob > F
Ethephon conc E	6	0.7262	0.90	0.5022
Replicates	3	0.3036	0.38	0.7695
Light/no light L	1	36.1607	45.00	0.0001
EL	6	0.5358	0.67	0.6769
Error	39	0.5545		
R-square	0.59			
C.V	72.75			
Mean	1.23			