ASPECTS OF FRUIT SIZE AND QUALITY IN CITRUS

BY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

Signature Date

SUMMARY

Fruit size can be a problem in 'Clementine' mandarin and 'Valencia' orange in the Western Cape region of South Africa. Small fruit is not only unacceptable to the consumer but is also more difficult and expensive to harvest. Means of alleviating this problem is to manage the crop load. Hand thinning trials were conducted to evaluate the effect of timing and severity thereof in enhancing fruit size. The benefits of enhancing large fruit size was obtained by a heavy-thinning (60% fruitlet removed) treatment, but the actual benefits were offset by a reduction of total yield. Yield was reduced up to 30% when heavy thinning treatments were applied. No effect on early or late treatments were obtained since the trial was conducted rather late (4 to 6 weeks after the physiological fruit drop period) to obtain the desirable results. However, better packouts are expected with thinning treatments since blemished fruit are also removed.

The effect of multiple 2,4-dichlorophenoxy propionic acid (dichlorprop) sprays were also evaluated on 'Nules Clemetine' mandarin. Comparing multiple sprays with a single spray, it was observed that more than one spray was no better in improving fruit size. The use of multiple dichlorprop sprays resulted in no additional yield reduction, while internal fruit quality was also not affected. The best results were obtained with dichlorprop at 50 mg. L^{-1}.

The use of dichlorprop with different surfactants was evaluated in both 'Clementine' mandarin and 'Valencia' orange. The fruit size was increased from 50 mg. L^{-1} up to 100 mg. L^{-1}. Yield was usually not affected but, where reductions were experienced, yield of large fruit (>55 mm) was not significantly affected. In the 'Valencia' orange trials, during the first year, dichlorprop was sprayed relatively late (fruit diameter: 19 mm). No fruit size, yield and internal fruit quality effects were observed. The following year, when early and late sprays

(fruit diameter at spray time = 8 and 12 mm, respectively) were evaluated, it was observed that late sprays had no effect on fruit size, whereas fruit size (48 fruit per carton) was significantly increased by early sprays. This implies that the dichlorprop effect on fruit size is during the early stages of fruit development, just after or during the late stage of the physiological fruit drop period. Yield was drastically reduced by up to 35%, which affected the actual kilograms of large fruit adversely in some treatments. Juice percentage was inconsistent and tended to be reduced by dichlorprop application. Dichlorprop tended to increase TSS slightly in year one and significantly so in year two, but did not affect the TSS:TA ratio. The use of Orchex mineral oils as surfactant at 150 mL. 100 L^{-1} was effective and seemingly allowed lower rates of dichlorprop to be used. Therefore, surfactants seemed to show potential in enhancing dichlorprop efficiency to reduce the application cost.

The dichlorprop-sprayed fruit was used to measure carotenoid content of the rind during the later stage of fruit development. Observations indicate that dichlorprop-sprayed fruit at 50 mg. L^{-1} obtained better carotenoid content in both 'Clementines' mandarin and 'Valencia' orange. Also, fruit exposed to light had higher carotenoid levels as compared to fruit shaded with brown paper bags. However, dichlorprop-sprayed fruit at 100 mg. L^{-1}, even though exposed to light did not show significant differences with unsprayed in both shaded and exposed conditions. Therefore, no consistent effect of dichlorprop was established on total carotenoid content of the rind.

OPSOMMING

Vruggrootte van 'Clementine' mandaryn en 'Valencia' lemoene kan 'n probleem wees in die Wes Kaap area van Suid Afrika. Klein vrugte is nie net onaanvaarbaar vir die verbruiker nie, maar is ook moeilik om te oes. Vrugdrag manipulasie is een manier om hierdie probleem te beheer. Hand uitdunningsproewe is uitgevoer om die effek van tyd en graad van uitdunning op vruggrootte te bepaal. Strawwe vruguitdunning (60% van vruggies verwyder) het groter vrugte tot gevolg gehad maar het gelei tot 'n verlaagde opbrengs. Opbrengs is tot soveel as 30% verlaag met die strawwe vruguitdunning. Vroeë en laat behandelings het egter geen effek gehad nie, aangesien die proewe te laat uitgevoer is (4 tot 6 weke na die fisiologiese vrugvalperiode) om die gewenste effek te verkry. Beter uitpak word verwag na uitdunning, aangesien vrugte met letsels ook verwyder word.

Die effek van veelvuldige 2,4-dichlorofenoksie-propioonsuur (dichlorprop) spuite is ook geëvalueer op 'Nules Clementine' mandaryn. Daar is geen verskil gevind tussen enkel en veelvuldige spuite in terme van vruggrootteverbetering nie. Die gebruik van veelvuldige dichlorprop spuite het nie tot 'n verlaging in opbrengs gelei nie, en interne vrugkwaliteit is ook nie beïnvloed nie. Die beste resultate is verkry met die 50 mg. L^{-1} dichlorprop behandeling.

Die gebruik van dichlorprop saam met verskillende benatters is op beide 'Clementine' mandaryn en 'Valencia' lemoene geëvalueer. Vruggrootte het toegeneem vanaf 50 mg. L^{-1} tot 100 mg. L^{-1}. Opbrengs was net in sekere gevalle verlaag en dan sonder 'n betekenisvolle verskil in groot-vrug produksie (>55 mm: Clementines). Tydens die eerste jaar van die 'Valencia' lemoen proef is die dichlorprop relatief laat gespuit (vrug deursneë: 19 mm). Daar was geen effek op vruggrootte, opbrengs en interne vrugkwaliteit nie. Tydens evaluasie van

vroeë en laat bespuitings die volgende jaar is waargeneem dat laat bespuitings geen effek op vruggrootte gehad het nie, terwyl vruggrootte (48 vrugte per karton) betekenisvol verhoog is deur die vroeë bespuitings (Vruggrootte voor bespuiting = 8 en 12 mm, onderskeidelik). Dit impliseer dat dichlorprop se effek op vruggrootte gedurende die vroeë stadiums van vrugontwikkeling is, net na of gedurende die laat stadiums van die fisiologiese vrugvalperiode. Opbrengs is drasties verlaag deur dichlorprop, tot soveel as 35% wat die kilogram groot vrugte negatief beïnvloed het in sommige behandelings. Sappersentasie was nie konsekwent affekteer nie, maar is verlaag deur die dichlorprop bespuitings. Dichlorprop het die TSS effens verhoog in beide jare, maar het geen effek gehad op die TSS:TA verhouding nie. Die gebruik van Orchex minerale olie as benatter was effektief by 150 mL. 100 L^{-1}, en laat klaarblyklik die gebruik van dichlorprop by laer konsentrasies toe. Dus, kom dit voor dat benatters die potensiaal het om die effektiwiteit van dichlorprop te verbeter wat lei tot 'n verlaging in toedieningskoste.

Die dichlorprop gespuite vrugte is gebruik om karotenoïed-inhoud van die skil te meet tydens die laat stadiums van vrugontwikkeling. Vrugte gespuit met dichlorprop teen 'n konsentrasie van 50 mg. L^{-1} het 'n beter karotenoïed-inhoud in beide 'Clementine' mandaryn en 'Valencia' lemoene tot gevolg gehad. Vrugte blootgestel aan lig het ook 'n hoër karatenoïed-konsentrasie gehad as vrugte wat met bruin papiersakke bedek was. Dichlorprop bespuite vrugte teen 100 mg. L^{-1}, alhoewel blootgestel aan lig, het geen betekenisvolle verskil gehad in vergelyking met onbespuite vrugte nie. Dus is daar geen konsekwente effek van dichlorprop op karotenoïde inhoud van die skil waargeneem nie.

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CONTENTS

1. INTRODUCTION

Fruit size is an important factor for any fresh fruit operation in citrus. Small fruit is not only expensive to harvest, and to handle in the packhouse, but also unacceptable to consumers, thus obtaining low prices.

There are a number of factors in production, which limits adequate and marketable fruit size. Many citrus varieties tend to bear small fruit size, especially alternate bearing varieties that produce heavy crops and small fruit during the "on" year (Greenberg, Hertzano & Eshel, 1992; Moss, 1972; Sutton & Harty, 1990; Wheaton, 1981). Also, 'Clementine' mandarins and 'Valencia' oranges, and other mandarin selections are genetically prone to bear small fruit (Guardiola, Garcia-Mari & Agusti, 1984). Despite the fact that larger fruit is achieved when it has more seeds than being seedless (Krezdorn & Phillips, 1967; Reuther *et al.*, 1969), most citrus varieties are planted in the absence of cross-pollination due to consumer demand for seedless fruit.

Small fruit size is a problem in certain 'Clementine' and 'Satsuma' mandarin selections in certain microclimates of the Western Cape Province of South Africa. The marked consumer preference for large fruit over smaller-sized fruits, particularly 'Valencia' oranges and 'Clementine' mandarins, also results in higher prices and returns to grower for larger fruit (Gilfillan, 1990). For this reason, the South African citrus industry is challenged to produce fruit that meet international market standards. The possibilities of increasing the average fruit size rest on either genetic improvement, or modification of cultural practices such as pruning and the use of plant growth regulators (Vanniere & Arcuset, 1992).

This review discusses the possible factors that may influence the final fruit size in citrus. This constitutes mainly two groups, namely, controllable and non-controllable factors. The grower has little or no means to manipulate climate. The former group comprises those factors that influence final fruit size before planting, such as rootstock and soil-related factors, to mention a few; and post-planting controllable factors include irrigation, nutrition and fruit load, and the grower has a direct impact on controlling these factors.

1.1. GROWTH AND DEVELOPMENT OF CITRUS FRUIT

Citrus fruit growth is divided into three growth stages which were first identified by Bain (1958) on 'Valencia' orange. Citrus fruit growth exhibits a typical sigmoid curve.

Stage I: Cell division period in which the number of cells in all tissues of the developing fruit increases to form the tissue of the mature fruit. The initial growth of fruitlets is characterised by an increase in the thickness of the pericarp as a result of cell division which lasts for 9 weeks after petal fall (Guardiola & Garcia-Luis, 2000). Stage I extends over 63 days (for 'Valencia' orange), from full bloom (mid September to mid November) in Southern Hemisphere (SH) (Bain, 1958). The increase in fruit volume is due to cell division.

Stage II: The first fruit growth is followed by a phase of cell enlargement which is characterised by vacuolization of the juice sacs, the marked increase in the size of the locules and a significant reduction in peel (pericarp) thickness (Guardiola & Garcia-Luis, 2000). This stage lasts 29 weeks in 'Valencia' orange, between mid November and mid-June (SH) and is the stage of maximum fruit growth (Gilfillan, 1987). There is a marked expansion of the tissues with cell enlargement, differentiation and the formation of intercellular spaces and spongy tissue in various parts (Gilfillan, 1990).

Stage III: This is the maturation stage, but fruit continues to increase but at a much lower rate (Gilfillan, 1990). This final stage is also characterized by a reduced rate of fruit enlargement and by numerous compositional changes, mainly increases in sugars and nitrogenous compounds and a continued decrease in the concentration of organic acids (Bain, 1958).

1.2. FACTORS AFFECTING FRUIT SIZE IN CITRUS

A. NON-CONTROLLABLE FACTORS

1.2.1. CLIMATE

The most important factor in citrus production areas is the climate or microclimate that determines which cultivar is best adapted. For instance, hot or warm areas are highly suitable for grapefruit production but not for 'Satsuma' and 'Clementine' mandarins. The components of climate that have been found to be important are ambient temperatures (daily maximum and minimum), solar radiation, day-length, humidity, and wind (Gilfillan, 1990). The growth rate of fruit shows a positive correlation with temperature, irrigation and sunshine hours, while it shows a negative correlation with the rate of evaporation. But none of these factors are independent; they interact with each other so that fruit growth is regulated completely (Zhang *et al.*, 1992).

1.2.1.1. Temperature

Temperature is a major factor influencing the final fruit size. Apart from photochemical reactions, general enzymatic reactions are affected by temperature. Increases in temperature can accelerate fruit growth (Zhang *et al.*, 1992). Thus, the effect of suitable temperatures on citrus fruit size can be explained better on fruit growth stages.

Stage I: The rate of cell division and enlargement is dependent on minimum and maximum temperature. Hence, maximum temperature during the flowering and the beginning of Stage I period improve fruit growth as long as it does not exceed 30°C. There is a rapid increase in fruit growth between 26°C and 30°C. Growth declines at maximum temperatures above 30°C and there is zero growth and possibly heat damage at 40°C (Gilfillan, 1990). Little growth occurs in all citrus organs below 13°C (Spiegel-Roy & Goldschmidt, 1996). Thus, fruit grown in the tropics are larger, but tend to senesce quicker (Davies & Albrigo, 1994).

Stage II: Fruit size is rarely limited by temperature that are too low during this period but rather by temperatures that becomes too high, thus restricting growth (Gilfillan, 1990). Data of Hilgeman, Tucker and Hales (1959) indicates that excessive temperatures (greater than 38ºC) depressed fruit growth in mid and late summer on 'Valencia' orange.

Stage III: No climatic effects on fruit size have been reported for this period (mid June to harvest for 'Valencia' orange). Fruit size continues to increase but at a lower rate (Gilfillan, 1990).

1.2.1.2. Sunlight hours

Warm sunny days and warm nights from July to October are associated with larger fruit size at harvest in Valencia orange (Gilfillan, 1990). At certain levels, when the duration of sunshine is abundant, photosynthesis rates are high. However, Ketchie and Ballard (1968) found that fruit grown under full exposure to the sun were smaller, weighed less and had less juice, higher total soluble solids (TSS) concentrations and more granulated carpels than fruit growing in the shade. Therefore, the relation between the fruit growth rate and the duration of sunshine is unsure, and differs from variety to variety (Zhang *et al.*, 1992).

1.2.1.3. Precipitation

Soil moisture has a great influence on fruit enlargement. Hence drought reduces fruit growth rate (Cooper *et al.*, 1963). Adequate rainfall and irrigation during Stage II (cell enlargement) significantly improves fruit size and juice content (Davies & Albrigo, 1994), although Spiegel-Roy and Goldschmidt (1996) emphasized that ample water supply during all fruit growth stages is of importance.

B.1. CONTROLLABLE FACTORS (PRE-PLANTING)

1.2.2. SOIL-RELATED FACTORS

1.2.2.1. Soil type

As rainfall and irrigation influence fruit size it is important to consider the soil type, which influences the infiltration, drainage and water holding capacity. Peng and Rabe (1998) reported that although not directly comparable, decreased irrigation at longer intervals in clay-loam soils tended to increase fruit total soluble solids (TSS) but reduced yield and fruit size compared to irrigation at shorter intervals in sandy-loam soil types.

1.2.2.2. Soil compaction

Root growth and tree performance can be limited by soil compaction which decrease O_2 transport to the root surface (Grant, 1993). Where roots are restricted by soil compaction, 80% of the roots occur in the top 0.5 m soil depth region (Abercrombie & Du Plessis, 1995). Alleviating soil compaction by deep ploughing on an established 'Navel' orange orchard resulted in a significant increase on fruit size starting from the second season of the experiment (Abercrombie & Du Plessis, 1995; Hoffman & Abercrombie, 1999). Percentage of large fruit mass increased linearly with non-compacted soil depth, which shows that a larger fruit size is favoured by a deeper non-compacted soil (Hoffman & Abercrombie, 1999). However, where soils are shallow and poorly drained, conventional deep ploughing can not be the solution but ridging is highly recommended (Abercrombie, 1996), since ridging improves the internal drainage of the root zone that is raised above the limiting layers in the sub-soil (Du Preez, 1985).

1.2.3. ROOTSTOCK

Fruit size is affected by choice of rootstock. Generally, lemon-type rootstocks such as rough lemon and *Citrus volkameriana* or those rootstocks that impart vigour to the scion, will produce larger fruit than less vigourous rootstocks (Davies & Albrigo, 1994). Less vigorous rootstocks such as 'Troyer' and 'Carrizo' citranges, sweet orange and 'Rubidoux' trifoliate (small flowered trifoliate) give intermediate size fruit. 'Cleopatra' mandarin, 'Pomeroy' trifoliate (larger flower) and 'Morton' citrange are associated with small fruit size (Gilfillan, 1990). Trees on trifoliate orange produce relatively small fruit of high quality in most areas. However, in Japan, trifoliate orange rootstock is used primary for 'Satsuma' and other mandarins. In the United States other scion varieties perform well on trifoliate orange (Krezdorn & Phillips, 1967; Salibe & Moreira, 1977).

1.2.4. SCION CULTIVAR SELECTION

Orange fruit size varies from cultivar to cultivar and these variations may be attributed to the genetic nature of the cultivar, but also to the climatic conditions and cultural practices (Sinclair, 1962). Various scion selections produce better fruit size than others. 'Delta' and 'Midknight' selections of 'Valencia' orange, for instance, tend to give better fruit size than 'Olinda' and 'Frost' nucellar selections (Gilfillan, 1990).

1.2.5. VIRUS LOAD

1.2.5.1. Citrus tristeza virus (CTV)

CTV, a member of the closterovirus group, is economically the most important citrus virus disease worldwide (Bar-Joseph *et al.*, 1979). Devastating losses can be encountered where sour orange is used as a rootstock for sweet orange, mandarins and more specifically to very sensitive grapefruit cultivars. However, citrus does not produce satisfactory in the presence of CTV, even if it is propagated on resistant rootstocks (Menechino, Mischan & Salibe, 1984; Van Vuuren & Van der Vyver, 1999).

The amount of tristeza stem pitting significantly affects some fruit quality characteristics. Fruit size and yield starts to deteriorate 7 to 8 years after infection and reduces the economic viability of 'Marsh' grapefruit to 10 to 15 years (Van Vuuren & Van der Vyver, 1999). Correlation studies done by Menechino *et al.* (1984) indicates that an increase in stem pitting rating is associated with a decrease in fruit diameter (fruit size), a decrease in percentage of acid, vitamin C content and an increase in the TSS, consequently resulting in higher total soluble solids (TSS): titratable acid (TA) ratios.

When the virulent strains are budded to sour orange rootstock, the trees are stunted, but otherwise appear normal in the nursery. The stunting becomes more apparent after 2 or 3 years in the field when fruit competition for carbohydrates lead to severe stunting and reduced fruit size (Davies & Albrigo, 1994). Recently, findings indicate that inoculating mild strains GFMS 12 and GFMS 35 on 'Star Ruby' and 'Nelruby' grapefruit, respectively, results in an increase of the small fruit percentage (Van Vuuren & Van der Vyver, 2000). Only LMS 6 (mild strain) showed less variation, pitting was found to be not severe, but had not been approved for the pre-immunization of grapefruit cultivars in South Africa.

B.2. CONTROLLABLE FACTORS (POST PLANTING)

1.2.6. MINERAL NUTRITION

Nutrition and fertilization practices always influence fruit quality. Thus, varying fertilizer rates and the ratio of elements is used commercially to change fruit quality depending on market demand. Nitrogen, phosphorus and potassium have the greatest influence on fruit size, provided that other elements are not severely limiting (Davies & Albrigo, 1994).

Nitrogen (N): N is an important constituent of protein and structural component of chlorophyll (Spiegel-Roy & Goldschmidt, 1996). It is essential for cell division and cell expansion. Also, N is responsible for vegetative growth, hence a deficiency results in yellowing of leaves. Therefore, as N levels increase, fruit size is increased through peel thickness and juice content thus decreases. Higher than optimum N levels can produce large, coarse, green fruit with low juice content (Davies, 1986). Du Plessis and Koen (1984) found that with an increased rate of N (up to 630 g N/tree) there was a decrease in fruit size. In other experiments, maximum fruit size was obtained with an N level of 2.2%. It can therefore, be concluded that an average N level in leaves of approximately 2.1% is ideal for maximum fruit size in 'Valencia' orange (Du Plessis, 1982).

Phosphorus (P): P is a component of nucleoproteins and phospholipids, and is involved in energy transfer. Low levels of P result in excessive abscission of old leaves and cause sparse bloom (Spiegel-Roy & Goldschmidt, 1996). However, high P levels reduce peel thickness and increase juice content whilst low P levels increase final fruit size (Davies, 1986). P seldom is a factor in fruit quality since visible P deficiency is rare in citrus (Reitz & Embleton, 1986).

Potassium (K): K is an important constituent of the fruit, plays a big role in translocation of carbohydrates and also acts as an osmotic agent in the opening and closing of stomata (Spiegel-Roy & Goldschmidt, 1996). A remarkable effect of low K is a drastic decrease on fruit size. Increased K levels generally increase fruit size and peel thickness in citrus (Davies, 1986). In Florida, Reese and Koo (1975) observed clear effects of K application on leaf K contents and fruit size. Du Plessis and Koen (1984), in South Africa, obtained the same results. They observed that KNO_3 sprays were effective in increasing fruit size. Bazelet, Feigenbaum and Bar-Akiva (1980) reported that an increase in leaf K by K application had clear effects on the soil K status, but failed to show significant effects on yield and fruit size. Results obtained by Dasberg *et al.* (1988) were rather different. They found no significant effect of K application on either yield, leaf K or fruit size. Soil K reserves were apparently sufficient in this case. The effect of K on citrus fruit size, though well documented, is not completely understood. Fruit size is a complex property determined by many factors which are more important than K application rate in order to improve fruit size (Dasberg, 1988).

Iron (Fe): The fruit response to the application of iron chelates is closely related to the degree of chlorosis. In severe cases the yields are drastically reduced by the chlorosis (Hilgeman, 1969). El-Kassas (1984) found that yield and fruit size were only improved by application of iron chelates on citrus showing moderate symptoms. However, leaf sprays and soil application of iron chelates to 'Navelina' orange trees indicated no significant difference in yield and fruit size (Legaz *et al*., 1992).

1.2.7. IRRIGATION

Water shortage will always be a limiting factor in citrus production (Yelenosky, 1991). Water deficit is generally associated with reduced productivity (Bradford & Hsiao, 1982). However, better irrigation can increase the number of fruits per tree substantially enough to cause an overall decrease in fruit size as a result of heavy crop load (Boman, Levy & Parsons, 1999). Therefore, irrigation timing and management has a major effect on citrus production.

Generally, it is accepted that adequate water supply is of major importance during flowering and fruit set. Deficit irrigation during this critical period may enhance abscission of flowers and fruitlets and a subsequent loss in yield, hence final fruit size is increased (Sardo & Germana, 1988). Water stress in Stage I of fruit growth mainly affects the number of fruitlets that are set and the number of cells formed in each fruitlet. Water stress in Stage II of fruit growth strongly affects the final fruit size because this is the period when the greatest rate of fruit growth takes place. Water stress during Stage III is not so crucial but can still affect fruit size adversely (Gilfillan, 1990).

The above mentioned knowledge led to regulated deficit irrigation, which has been having good results on both water use efficiency and citrus fruit quality (Torrecillas, Ruiz-Sanchez & Domingo, 1993). In some other studies, fruit size was not significantly affected by regulated deficit irrigation (Castel & Buj, 1990), but decreased the puffiness of 'Satsuma' mandarin in areas of high rainfall in Japan (Kuriyama *et al.*, 1981).

1.2.8. TREE COMPLEXITY

1.2.8.1. Pruning

Pruning is a practice that has been considered absolutely necessary to obtain high yields and good fruit quality (Reitz & Embleton, 1986). Elimination of branches result in good light distribution to the lower parts of the canopy (Davies & Albrigo, 1994) and decreased number of fruits in the tree (Zaragoza *et al.*, 1992). These two factors contribute to production of larger fruit at harvest. If pruning could aim to remove wood of poor bearing potential, the remaining fruit can grow larger due to less competition (Krajewski, 1996). Hence there is a strong linkage between intensive pruning, yield and fruit size (Oren, 1988).

Considering methods of pruning that had been conducted in most citrus cultivars, variable results had been obtained. Moore (1957) found in very old 'Valencia' and 'Navel' orange orchards, that moderate pruning had no effect on yield, but slightly increased fruit size. Also, hedging of 20 year-old 'Valencia' orange trees in Cuba increased fruit size and yields over unpruned trees. Most recently Morales, Davies and Littell (2000) indicated that skirting

(removal of low hanging shoots) did not have an effect on fruit size improvement on 'Orlando' tangelo.
Light shaping to an open vase, significantly increased fruit size by 4 mm in 'SRA 89 Clementine' mandarin done in September (Gilfillan, 1995). Zaragoza *et al*. (1992) observed a 2 mm difference in fruit size compared with unpruned 'Clausellina Satsuma' mandarin trees pruned at fruit set. In case of old 'Clementine' mandarin trees, pruning to "skeletoning" (only main branches left after pruning) partially solved the tendency of gradually bearing smaller fruit (Oren, 1988). However, for a year following the pruning, the trees did not bear any fruit, then for 3 to 4 years the fruit is adequate with a gradual deterioration in size. Therefore the entire procedure has to be repeated. In another study, Hilgeman (1972) pruned old 'Marsh' grapefruit trees lightly, moderately and severely. Results showed a rapid yield recovery, improved quality, fruit size and maintenance of smaller tree size with severely pruned trees. However, Raciti *et al*. (1981) observed no statistically significant results in the average fruit size, even with severely pruned lemons or drastically pruned oranges.

Economic forces also must be considered to evaluate production cost, especially those of pruning, which represent 20% of the total cost of cultural practices (Reitz & Embleton, 1986). Sometimes benefits obtained from pruning do not compensate for the decrease in yield caused by pruning (Zaragoza *et al*., 1992). However, if pruning is done timeously and correctly, large areas can be handled with relatively low labour inputs (Joubert *et al*., 1999).

1.2.8.2. Girdling

Stem girdling has a good effect in citrus on yield and fruit size (Cohen, 1984; Rabe & Van der Walt, 1992) as well as quality and ripening date (Hochberg, Monselise & Costo, 1977). Citrus productivity and tree response to girdling depends on many factors, such as girdling date, girdling procedure and technique (such as girdling width and position, i.e. trunks or branches), different cultivars and possibly even climatic differences between countries or regions (Peng & Rabe, 1996).

Girdling severs the phloem vascular tissue thereby preventing translocation of photosynthates from the source to sinks located below the girdle until the wound heals. Thus, girdling has an indirect effect of reducing sink size and increase the amount of photosynthates available to fruits and other active meristems above the girdled region (Krezdorn & Brown, 1970; Van der Poll, Miller & Allan, 1991). Winter girdling increases the number of buds sprouting in spring, hence fruit size is negatively affected (Cohen, 1981). Spring girdling increases fruit yield through increasing fruit set (Erner, 1988). Autumn girdling reduces the number of buds sprouting and markedly increase flowering, hence reduces fruit size (Cohen, 1981); spring girdling reduces fruit size while summer girdling increases fruit size (Cohen, 1984). However, Peng and Rabe (1996) found no effect on fruit size distribution with girdling treatments under both deficit and normal irrigation.

Girdling also imposes several effects on the plant, including direct injury, and as such it elicits several responses (Cohen, 1981). Weak trees should not be girdled and girdled trees must be well irrigated (Cohen, 1984). Thus, it is best to girdle only two thirds of the citrus tree, though sometimes not effective, thereby reducing the danger of root injury.

Due to the labour intensity of girdling and the contradicting results obtained with this practice only few growers use girdling to increase fruit size (Davies, 1986).

1.2.9. FRUIT LOAD

Physiological fruit drop is a self-thinning mechanism, which adjusts the number of reproductive units on the tree (Goldschmidt *et al.*, 1992). Some cultivars do not abscise enough fruitlets during the physiological fruit drop period and therefore should be thinned to prevent fruit overload and to enable the remaining fruit to reach adequate and marketable size.

1.2.9.1. Thinning

Fruit thinning is the removal of fruitlets in heavy fruit set situations to avoid alternate bearing and to reduce the intersink competition of fruitlets, allowing the remaining fruits to grow larger. This practice is performed to increase fruit size, but its benefit depends on the thinning procedure (light or heavy thinning) and timing used (Guardiola & Garcia-Luis, 1997). Also, fruit size is increased with the number of leaves available to supply carbohydrates to the growing fruit. Fruit and flowers with one or more leaves on the shoot supporting it, produce faster growing and eventually larger fruit at harvest (Gilfillan, 1990). There are two types of thinning that had been identified, namely mechanical (hand) and chemical (hormonal) thinning aimed at achieving a high leaf:fruit ratio. Increasing leaf to fruit ratio enables the remaining fruit to grow larger but at the expense of a possible yield reduction.

1.2.9.1.1. Hand thinning

Hand thinning had been successful in improvement of fruit size in a number of varieties in citrus, such as, 'Washington Navel' orange (Parker, 1934), 'Kinnow' mandarin (Hilgeman, True & Dunlap, 1964), and 'Valencia' and 'Tomango' oranges (Gilfillan, 1987; Rabe, 1991). For best results, hand thinning is carried out in late January or early February (S.H) after fruit set to give a profitable combination of fruit size and crop load (Gallasch, 1998). This late performance of hand thinning is based on the knowledge that fruit is not always a strong sink at the early stage of development. According to Gilfillan (1987), hand thinning of 'Valencia' and 'Tomango' oranges is carried out on 30 to 50 mm fruit diameter. Fruit size is improved when 20 to30% of fruit are removed.

Thinning must be severe, for the outcome of reduction in competition to be evident (Zaragoza *et al.*, 1992). Hilgeman *et al.* (1964), Rabe (1991) and Zaragoza *et al.* (1992) found that there was a good relationship between the severity of thinning and fruit size improvement for 'Kinnow' mandarin, and both 'Tomango' and 'Valencia' oranges and 'Clausellina Satsuma' mandarin, respectively. Heavy thinning leaves one fruit to 12 leaves, thus more photosynthates are available for fruit development in 'Satsuma' mandarin (Sutton & Harty, 1990). Correspondingly, the most severe thinning treatments in 'Satsuma' mandarin reduced total yield by an average varying only from 10 to 14%. However, before fruit hand thinning be considered for improving fruit size it should be ascertained that the only factor which is limiting adequate fruit size is excessive fruit load and not other problems (Rabe, 1991).

The high labour requirement for hand thinning can render this practice impractical and uneconomical (Gilfillan, 1987).

1.2.9.1.2. Chemical thinning: Auxins and different plant growth regulators

The effect of the application of synthetic auxins on fruit size is the result of at least four independent effects on fruit development (Guardiola, 1988), namely: (i) a transient reduction in fruitlet growth rate that reduces the final fruit size; (ii) a stimulation of fruitlet abscission linked to the synthesis of ethylene (thinning effect), which increases fruit size by reducing intersink competition; (iii) a direct stimulation in late fruit growth rate; or (iv) an increase in sink strength of developing fruitlets.

The final effect of these applications on fruit size and yield is the result of the interaction of these effects, which depend on the nature of the auxin (Guardiola & Garcia-Luis, 2000), the time of application, cultivar used and the concentration applied (Ortola, Monerri & Guardiola, 1991).

(a) Ethephon

The production of alternate heavy and light crops is a major problem with 'Valencia' orange trees in Australia (Moss, 1972). One feature of this phenomenon is the development of small fruit during the heavy crop year. Plant growth regulators are required to reduce fruit numbers to improve final fruit size. An ethylene-releasing substance, Ethrel® or ethephon, is commercially available as a pre-harvest spray. Spray application of ethephon around the normal fruit setting period (November drop), successfully thinned several heavy set citrus cultivars (Hield, Burns & Coggins, 1962) and increased fruit size at harvest in 'Valencia' and 'Washington Navel' oranges in California (Stewart, Hield & Brannaman, 1952). Also, ethephon sprayed on fruitlets of 10 mm diameter in 'Wilking' mandarin increased fruit size significantly without reducing yield relative to the hand-thinned control (Galliani, Monselise & Goren, 1975).

Hutton (1992) identified an optimum concentration of ethephon as a thinning agent for the practical control of alternate bearing in 'Valencia Late' oranges. A single, high volume spray of ethephon at 42 to 60 ppm applied in a heavy set year at 6 to 8 weeks post bloom when the fruit size was 10 to 15 mm in diameter induced a 15 to 20% reduction in fruit number. With internal fruit quality unaffected, thinning allows the remaining fruit to grow larger (Hutton, 1992). Gilfillan (1987) found an average increase of large-fruit yield (counts 56, 72 and 88) of 30.8 kg/tree when compared to the untreated control for a loss of 23% of the total yield (65.9 kg/tree). However, ethephon induces defoliation (Morton *et al.*, 1978), and is not recommended on certain cultivars such as 'Navel' orange, even at low spray concentrations (Gilfillan, 1992).

(b) Naphthalene acetic acid (NAA)

NAA has been successfully used to increase fruit size in 'Kinnow'mandarin (Hilgeman *et al.*, 1964; Brar, Minhas & Kaundal, 1992), 'Dancy' tangerines (Wheaton & Stewart, 1973), 'Satsuma' mandarin (Hirose, 1981), and 'Star Ruby' grapefruit (Greenberg *et al.*, 1992). NAA's success is mainly owing to its thinning effect. Although a direct effect of this compound on fruit growth has been reported (Guardiola, Almela & Barres, 1988; Ortola, Monerri & Guardiola, 1997), it could not be separated from the thinning effect since the application of this compound always resulted in a decrease in the number of fruits finally cropped. Application during flowering or after fruit set causes significant thinning (Hirose, Iwagaki & Suzuki, 1978), whilst application after November drop does not affect the fruit count (Ortola *et al.*, 1997). In regular bearing cultivars, NAA effect of increasing the fruit size is offset by a reduction in yield (Ortola *et al.*, 1991).

(c) Ethychlozate

Ethychlozate (ethyl 5-chloro-1H-3-indazolylacetate, Figaron®) is a registered thinning agent in Japan, and is successfully used in 'Satsuma' mandarin (Iwahori, Tominaga & Oohata, 1986; Iwahori, 1990). This synthetic auxin formulation when applied after November drop, unlike other thinners, influences most fruit quality factors such as increasing Brix, advances colouration and accelerates maturation (Hirose, 1981). Kamuro and Hirani (1981) observed that fruit sprayed with ethylchlozate is more uniform in size at harvest and alternate bearing incidence is lowered. The effect of this plant growth regulator is owing to thinning and has no direct effect on fruit growth.

(d) 2,4-dichlorophenoxy propionic acid (2,4-DP)

Applying the butylglycol ester of 2,4-DP (Corasil E®) just after physiological fruit drop successfully increased final fruit size of 'Clementine' mandarin (Agusti *et al.*, 1994; Koch, Theron & Rabe, 1996; Miller, Bird & Maritz, 1999; Rabe, Koch & Theron, 1995) and 'Fortune' mandarin (El-Otmani *et al.*, 1993). In comparison with the majority of synthetic auxins, 2,4-DP does not thin fruit severely. Therefore yields are not affected by 2,4-DP application because fruit load is not reduced significantly (Agusti *et al.*, 1994). However, thinning can be marked when 2,4-DP is applied very early or when applied at high concentrations (Vanniere & Arcuset, 1992). This indicates that date of application and concentration are crucial factors for satisfactory results.

El-Otmani *et al.* (1993) discovered that 2,4-DP application on 'Fortune' mandarin had an increase in fruit size. The increase in fruit size was linear with an increase in spray concentration up to 100 mg. L^{-1}. This increase was due to treatment being associated with an absolute, but not relative, juice content with no consistent effect on peel thickness. This suggests that the auxin affects mostly endocarp growth parameters rather than juice content or peel weight, which are dependent on fruit size only. Due to treatment of 2,4-DP, locule sizes are increased as a result of an increase in vesicle size, but not vesicle number. This stimulation effect of 2,4-DP on growth of the endocarp is explained by the fact that the treatment was applied at the stage of cell expansion (after November drop) rather than that of cell division (Bain, 1958). Therefore 2,4-DP stimulates fruit growth directly at the beginning of the cell enlargement stage. Also, Agusti, Almela and Aznar (1992) observed that if the treatment was delayed after November drop, there was no effect on fruit size was obtained, meaning that cell enlargement was less.

(e) 2-4 dichlorophenoxy acetic acid (2,4-D)

Though 2,4-D showed good results in improving citrus fruit size and is inexpensive, it can be phytotoxic (Salisbury & Ross, 1992). This synthetic auxin has a direct stimulatory effect on fruit growth. Early application of 2,4-D in 'Satsuma' mandarin increased fruit size (Guardiola & Lazaro, 1987). Greenberg *et al.* (1992) observed no effect on fruit size of 'Star Ruby' grapefruit treated with 2,4-D and KNO_3, and rather increased yields. Miller *et al.*

(1999) reported that 2,4-D and KNO_3 had the greatest effect on increasing fruit size in 'Clementine' mandarin, with significantly larger fruit in all size categories from 55 to 72 mm and significantly less fruit of 51 mm diameter and smaller.

(f) 2,4,5 trichlorophenoxy acetic acid (2,4,5-T)

The effects of 2,4,5-T on fruit size have been determined in the parthenocarpic 'Satsuma' mandarins (Guardiola *et al.*, 1993). They observed that final fruit size was increased with late application (42 days old fruit) of 2,4,5-T, and its specific effect was on the enlargement of juice vesicles (Guardiola *et al.*, 1993). When mixed with 2,4-D, 2,4,5-T had a direct stimulation on fruit growth at 75 mg. L^{-1} without thinning (Guardiola & Lazaro, 1987). This combination reduced peel thickness and puffiness despite the large fruit these auxins produced. 2,4,5-T was observed to increase fruit growth if applied after November drop and its effect on fruit size was marked by reducing fruitlet growth and stimulate late fruit growth, hence increased fruit size at harvest (Guardiola *et al.*, 1988).

(g) 3,5,6-trichloro-2-pyridyl-oxyacetic acid (3,5,6-TPA)

The plant growth regulator 3,5,6-TPA can be used to improve final fruit size in citrus. Early applications, just after November drop increased fruit size via a strong fruit thinning on 'Fina Clementine' mandarin (Agusti *et al.*, 1995). Thinning increased with an increase in 3,5,6-TPA concentration up to 10 mg. L^{-1} and resulted in a considerable reduction in fruit competition and in yield. Delay in treatment reduced fruit thinning, with the auxin directly improving fruit size, with little effect on yield (Agusti *et al.*, 1995).

1.3. CONCLUSION

As fruit size can be a limiting factor in citrus production, it must be studied and well understood. A single factor may be the reason for the small fruit size or there may be a combination of factors in a particular orchard and this requires good management skills. Also, whilst focusing on improving fruit size, other quality factors must be considered because they might be affected as well. For instance, when high levels of N are applied to achieve adequate size, colour break might be delayed and that would delay the marketing of the fruit. Also, seedless fruit which is preferred by the consumers might yield small fruit.

Practices such as hand thinning and girdling are labour intensive. Therefore, a grower must consider the effectiveness of the practice in relation to the labour cost and availability. Some practices reduce total yield (thinning, pruning, and the use of some plant growth regulators). Every practice is meant to improve fruit size and returns. Therefore, financial implications of each practice should be considered before any decision is taken.

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2. THE ROLE OF SURFACTANTS IN SPRAY APPLICATION

The term surfactant is a shortened form of "surface active agent" and refers to a variety of chemicals with several uses. Their importance can never be underestimated in agriculture since they are widely used in agronomy, dairy and pasture, forestry, turf grass management, horticulture, entomology and plant pathology. Surfactants included in agricultural sprays markedly affect performance by producing smaller droplets, improving wetting and influencing retention (Thomas & Hall, 1979). These chemicals, also called wetting agents or spreaders, physically alter the surface tension and consequently reduce the contact angle between the air-water and water–oil interfaces (Swietlik & Faust, 1984). Surfactant solutions produce smaller contact angles (Schonherr & Bukovac, 1972), with complete wetting best achieved when the contact angle is zero (Swietlik & Faust, 1984).

However, the effectiveness of surfactants cannot only be attributed entirely to a lowered surface tension because when the surfaces are naturally and readily wettable, the lowering of the surface tension may decrease the spray retention (Sargent, 1965). In addition, too much surfactant can cause excessive run-off or deposit loss, thus reducing the spray efficiency.

2.1. SURFACTANT FORMULATION AND MODE OF ACTION

Surfactants are classified by the way they ionize the solution. A surfactant with a negative charge is anionic, one with a positive charge is cationic and the one without an electrical charge is said to be non-ionic. Depending on the nature of the active ingredient, surfactants are water-soluble and have good emulsifying, dispersing and wetting properties. Anionic surfactants are most effective because they can be water-soluble soaps and water-soluble synthetic surface-active compounds. Cationic surfactants can be phytotoxic, so they should not be used as stand-alone surfactants. Selection of a wrong surfactant can reduce the efficacy and injure the target plant (Hock, 1994). Different surfactants contain different amounts of active ingredients. It is therefore recommended to use only the type directed on the chemical label.

The surfactant molecule comprises hydrophilic and hydrophobic ends. The hydrophobic components are attracted to each other. Therefore, the hydrophobic end of the surfactant molecule attaches to the hydrophobic coating of the target surface. This leaves the hydrophilic end of the surfactant molecule facing outwards, providing a site for water and spray molecules to attach (Zhang & Basaran, 1997).

2.2. THE INFLUENCE OF SURFACTANT IN SPRAY EFFECTIVENESS

2.2.1. Stomatal penetration

Openings in the epidermis such as stomata are possible sites of entry for foliar applied chemicals. The cuticle is a barrier to penetration, but is not impenetrable (Dybing & Currier, 1961). Also, since many plant leaves are hairy and waxy they tend to repel spray droplets, thus reducing spray efficiency. Pure water will stand as a droplet, with a small area of contact with the waxy leaf surface, but when containing a surfactant, the water droplet will spread in a thin layer over a waxy leaf surface (Miller & Westra, 1995).

Surfactants increase penetration of leaf cuticles by causing swelling because of greater wetting, thus improving stomatal pore penetration. Grieve and Pitman (1978) compared the uptake of salt solutions containing surfactants through stomatous and astomatous leaf surfaces of grapefruit (*Citrus paradisi*). They observed no effect of surfactant on astomatous surfaces, though uptake through stomatous surfaces was enhanced three-fold by surfactants. Also, stomatal penetration of NAA and silver nitrate into pear (*Pyrus communis* L. cv. Bartlett) leaves was induced with addition of surfactants (Greene & Bukovac, 1974). A rapid increase of uptake was observed in open stomata.

2.2.2. Foliar absorption

When soil conditions favour nutrient fixation or insolubility of compounds of the nutrient, foliar application produces the most efficient plant response to these ions, especially those required in low amounts by the plant. Various spray additives and surfactants have been used to increase the rate and amount of nutrient ion absorption.

The results of using wetting agents on nutrient uptake have been inconsistent. Calcium foliar uptake can be affected by differences between wetting agents. Hanekom and De Villiers

(1977) showed that six out of seven wetting agents actually reduced the amount of Ca absorbed, probably by enhancing run-off. Conversely, low concentrations of Agral 90 (0.006%) significantly increased uptake of Ca relative to the control (Wooldridge, 1999). Fat-sugar-derived surfactants were examined by Cantliffe and Wilcox (1969) on foliar uptake of phosphorus (P). They observed that the 0.5% concentration of wetting agents used, was more effective than the other two concentrations (i.e. 0.1% and 1.0%).

However, Henning and Coggins (1988) observed no relation between surfactant concentration and surface tension or area wetted due to inactive ingredients contained in surfactants used. Therefore, surfactant concentration may not be the only determining factor of spray effectiveness.

2.2.3. Spray stability

Another important role of surfactants is the dispersion of solid particles in its function as a colloidal particle stabilizer. The stabilizing mechanism can involve either electrostatic repulsion with ionic surfactants or steric stabilization with nonionic surfactants. In both cases, to provide stabilization, the surfactant adsorbs to the particle surface with the hydrocarbon chain towards the solid leaving the head group towards the aqueous phase (Ottewill, 1979). In modern spray dosages, chemical suspension plays a very important aspect in the uniform stabilization of a chemical, hence achieving constant dosage (Rendall & Smith, 1979). Also, surfactants are important components of chemical spray formulations which can be mixed as an in-tank addition to improve the effectiveness of the active ingredient (Chow & Grant, 1992).

2.2.4. Rainfastness

Weather conditions at the time or following application of a spray chemical can influence its effectiveness. Cool and cloudy weather and also heavy rain following the spray application will wash away the chemical and therefore reduce its effectiveness (Lantagne, Koelling & Dickmann, 1992).

Without proper wetting and spreading, spray droplets often run off or fail to adequately cover the leaf surface. Surfactants cause the spray droplet to stick better to the plant leaves even after rain (Lantagne *et al.*, 1992). However, not all surfactants are equal in degree of rainfastness. Surfactants that provide rapid rainfastness includes esterified seed oils, organo-silicates and most nitrogen-surfactant blends (Miller & Westra, 1995). The effectiveness of surfactants on rainfastness was evaluated by Balneaves and Popay (1992) who discovered that surfactants are beneficial in enhancing spray effectiveness with rain after application. Furthermore, they observed that 0.5% surfactant concentration was essential for control where rain occurred within two hours of application.

2.3. THE EFFECT OF SURFACTANTS ON PLANT MATERIAL

Sometimes surfactants are advertised and claimed to be universally effective to every type of plant material. Such claims may be misleading. Sometimes, whilst commonly used materials are not phytotoxic, excessive rates of nonhorticultural surfactants can result in negative effects on plant growth. For some surfactants, phytotoxic effects are observed with severe leaf curling and discolouring (Cantliffe & Wilcox, 1969). Some surfactants may damage cellular membranes, or may precipitate inorganic salts (Swietlik & Faust, 1984). Blue-green conifers may temporarily lose their blue colour due to the use of surfactants in herbicide sprays, especially with crop oil concentrates. Therefore, caution is advised when using a wetting agent with particularly sensitive crops, and testing of new products on a small sample of the crop is highly recommended (Blodgett, Beattie & White, 1995).

2.4. CONCLUSION

'Spray and pray', is an old saying that's still true nowadays because there are a number of factors influencing spray effectiveness. The use of surfactants minimize some barriers which limit spray effectiveness. Not all chemical sprays require a surfactant be added because some are already containing these compounds. Therefore, labels must be read and the recommendations provided must be followed.

Generally, surfactants enhance the uptake of foliar sprays by reducing the leaf surface tension and therefore provide uniform distribution of sprayed chemicals along the leaf surface, improve retention and possibly absorption. Wetting agents can be used commercially to improve the spray efficiency. Unfortunately, some surfactants are not yet registered. The prevailing weather conditions such as rain after spray application can reduce spray effectiveness by causing run-off, but this effect can be minimized by application of surfactants. Wetting agents are not insurance for the spray application, so growers must use recommended surfactants and concentrations since effectiveness of some surfactants may decrease with time. In addition, surfactants can be phytotoxic to some plants at certain concentrations.

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3. BIOCHEMICAL CONSTITUENTS OF THE CITRUS RIND

Citrus fruit is comprised of an outer coloured exocarp (flavedo) and an inner white spongy mesocarp (albedo) and an edible portion (endocarp) (Whiteside, Garnsey & Timmer, 1988). The peel (rind) consists of the flavedo and the albedo (Baldwin, 1993). The flavedo is the pigmented portion of the peel and comprises of the cuticle, epidermal and sub-epidermal cells, oil glands and associated enveloping cells, and vascular bundles (Petracek, Hagermaier & Dou 1999). During the early stages of fruit development, the flavedo is a dark green, photosynthetically active tissue, with a relatively small number of stomata (Goldschmidt, 1988). During the change from the original green colour to the colour typical of oranges, chlorophyll content in the exocarp decreases and carotenoids accumulate. The different biochemical constituents of the rind affecting fruit qualities such as colour, keeping quality and physiological disorders are discussed in this review.

3.1. RIND PIGMENTS

3.1.1. Chlorophyll

Chloroplasts consist of two chlorophyll pigments namely chlorophyll a and b, of which the former is a major pigment and the latter is accessory. However, both chlorophyll a and b gradually decrease near colour break stage and disappear completely in the mature fruit (Gross, 1981). The rate at which chlorophyll b is degrading is faster than that of chlorophyll a (Hsu, Lu & Yang, 1995; Shimowaka, 1990). Gibberellin treatments and high nitrogen levels delay the normal loss of chlorophyll in maturing fruit. However, chlorophyll breakdown is accelerated by exogenously-applied ethylene or ethylene-releasing compounds such ethephon (Gilfillan, 1992) and ethychlozate (Iwahori, Tominaga & Oohata, 1986; Tominaga, Kozaki & Iwahori, 1995). The forced chlorophyll degradation is not synchronized with carotenoid synthesis, but it is controlled by other factors, such as the accumulation of chlorophyllide a (Pons *et al.*, 1992).

If the fruit is kept on tree during the summer months, chlorophyll returns to the rind. This is referred to as regreening (Saks *et al.*, 1988). The tendency of citrus fruit to regreen have been observed in lemons (Wrischer *et al.*, 1986) and 'Valencia' oranges (Caprio, 1956). Ultrastructural studies have shown that regreening results from the reversion of chromoplasts, rather than from the formation of new chloroplasts (Saks *et al.*, 1988). Since the fruit is capable of photosynthesizing, the photosynthates remain in the peel (Baldwin, 1993).

3.1.2. Carotenoids

Other important pigments in citrus rind are carotenoids that are red, orange, and yellow lipid-soluble pigments found embedded in membranes of chloroplasts and chromoplasts (Bartley & Scoinik, 1995). Their colour is masked by chlorophyll in photosynthetic tissues and unmasking is during the disappearance of chlorophyll.

Carotenoid content of grapefruit peel is relatively low, with the colourless carotenoids, phytoene and phytofluene, making up to 70% of the total (Baldwin, 1993). Also, in lemons, 25% of the peel carotenoids are colourless. Hence, the presence of red carotenoids reticulataxanthin and 3-hydroxysintaxanthin, and ß-carotene result in 'bronzing' of lemon (Baldwin, 1993). The accumulation of these colourless precursors in white grapefruit stems from the genetical block that hinders further dehydrogenation steps leading to coloured carotenoids. Hence, in pink and red grapefruit cultivars it is evident that the genetic block has been removed. Lycopene is responsible for red colour in 'Ruby Red 'grapefruit (Sandler, Davis & Dezman, 1990). Its concentration in tissues of 'Chandler' pummelo at harvest is high in warm climates, but rather low in cool climates.

During photosynthesis carotenoids play a vital role in photoprotection against harmful photo-oxidation (Bartley & Scoinik, 1995). Carotenoids operate in the photosynthetic system as light harvesters and act directly as shielding pigments in the ultraviolet range of the spectrum (Lichtenthaler, 1987). Carotenoids, also have anti-oxidant function which protects cells from free radical damage by irradiating energy transfer which is later released in the form of heat.

Goldschmidt (1988) reported that carotenogenesis requires light and is promoted by low temperature and ethylene (Eaks, 1977). High light intensity promote carotenoid content of the peel, hence fruit growing in shaded areas tend to be paler (Coggins & Hall, 1975), and therefore pruning had been successful to avoid shading in the inside growing fruit (Tibshraeny, 1995). However, light causes an increase in rind temperature, which in turn cause regreening in 'Valencia' orange (Caprio, 1956; Huff, 1983). Also, stylar-end breakdown of 'Tahiti' lime is induced by high temperatures in fruit exposed to the sun whether attached or detached (Davenport & Campbell, 1977).

Warm nights, particularly (25°C to 30°C) have stronger effect on carotenoid accumulation than day temperatures. Stewart and Wheaton (1971) reported that the quantity of ß-citraurin was reduced at high temperatures. Therefore, it is not surprising when high temperature leads to chlorophyll build-up after the orange-yellow colour had already been obtained (Coggins, Hall & Jones, 1981).

3.2. PLANT HORMONES

3.2.1. Ethylene

The gaseous plant hormone ethylene (C_2H_4) regulates many aspects of plant growth, development and senescence. Both the albedo and flavedo are capable of producing trace amounts of ethylene via the ACC pathway (Baldwin, 1993). Ethylene production cause changes in colour, texture, aroma and flavour by interacting with other plant hormones such as auxins, gibberellic acid (GA), cytokinins, abscisic acid (ABA) and the expression of genes which are responsible for a variety of processes, including a rise in respiration (Wills *et al.*, 1989). The exact mode of action of ethylene in rind degreening has not been determined, but it is generally accepted that, on-tree degreening happens when night temperatures are sufficiently low for chilling to cause mild injury to the rind. This stimulates the production of stress-related wound ethylene (Cooper, Henry & Waldon, 1969). Sometimes the premature loss of chlorophyll and colouring of the rind may indicate that the fruit has been injured by certain pests or diseases that induce ethylene production (Whiteside *et al.*, 1988).

According to the hypothesis of McMurchie, McGlasson and Eaks (1972), citrus lacks a ripening-associated autocatalytic rise in ethylene production. But, this does not rule out a role for the small but detectable amounts of endogenous ethylene in the regulation of ripening processes. In citrus, silver and norbornadiene (NBD) strongly inhibited the loss of chlorophyll from green harvested 'Shamouti' orange fruit (Goldschmidt, Huberman & Goren, 1993), unlike in strawberry where inhibitors of ethylene biosynthesis did not interfere with the accumulation of anthocyanins. Furthermore, the rapid, spontaneous loss of chlorophyll from green harvested 'Shamouti', as compared with 'Valencia' orange fruits, is correlated with several fold higher levels of ethylene evolution (Goldschmidt & Galily, 1995; Goldschmidt, 1998).

3.2.2. Gibberellins

Young orange and lemon fruit were reported by Khalifah (1967) to contain gibberellin-like substances which were identified as GA_1 and GA_9. Thereafter, Erner, Goren and Monselise (1976) found that both the flavedo and albedo of rough 'Shamouti' orange fruit contained more gibberellin-like substances than smooth-peeled fruit. According to these findings they concluded that maturing fruit has less gibberellin-like substances than younger fruit. Upon treatment of the maturing fruit with ethylene, gibberellin components decrease rapidly (Goldschmidt & Galily, 1974). Hence, in regreening of 'Valencia' orange, gibberellin-like substances was found to build up (Rasmussen, 1973). With this knowledge, exogenous gibberellins had been used extensively to delay the peel senescence and extend the harvest season (Brown, 1993). Also, creasing, a physiological disorder, can be prevented by gibberellin sprays during the growing period, 8 to 10 months before its appearance in 'Valencia' orange (Monselise, 1977).

3.2.3. Auxins, cytokinins and abscisic acid

Auxins can only be found in very young fruitlets (Igoshi *et al.*, 1971). Fruits harvested two months after full bloom were found to contain no auxins. Hence synthetic auxins play a major role in stage two of fruit growth. A synthetic auxin, 2,4-D, causes a delay in chlorophyll degradation and has little or no influence on carotenoid content (Coggins & Jones, 1977).

Since cytokinins are involved in the cell division stage of the fruitlet it is evident that when fruit approaches maturity, cytokinins activity would be low (Monselise, 1977). Rough peel contained more cytokinins than smooth-peel as well as flavedo more than the albedo (Goldschmidt, 1976).

Endogenous abscisic acid (ABA) content increases during the course of colour development and reaches a maximum at colour break (Richardson & Cowan, 1995). Thereafter, ABA declines due to enhanced catabolism and formation of ABA-conjugates (Harris & Dugger, 1986).

3.3. OIL GLANDS

The special cells located in the flavedo that produce terpenes and oils form the oil glands. The oil gland cavity forms schizogenously and/or lysogenously by the separation and/or lysis of cell walls of the central cells (Baldwin, 1993). Also, oil glands are toxic to the surrounding living cells and are the cause of oleocellosis. Oleocellosis is also caused by picking fruit whilst wet and subsequent rupturing oil cells (McCornack, 1970). Rupture of oil glands may also result in necrosis of the adjacent epidermis, inducing the formation of irregularly shaped yellow, green or brown spots in which the oil glands of the skin protrude prominently because of slight sinking of the tissue between them (Murata, 1997). Flavedo epidermal cells produce cutin and waxes and contain actinocytic type stomates.

3.4. CONCLUSION

The rind constituents affect the external appearance and the keeping quality of the fruit. Temperature is one of the factors that play a major role in determining the final product. High temperatures towards harvest hinder the proper colour development of the rind. Hence at lower temperature (less than 12.8°C) chlorophyll is degraded and carotenoids accumulate. However, chlorophyll breakdown can be induced by ethylene or ethylene-releasing compounds but that is not a guarantee for the carotenoid accumulation.

Studies of hormones during active growth indicated that some hormones such as gibberellins or cytokinins are essential in the early stage of fruit development. However, levels of these hormones towards harvest affected rind texture and colour development adversely. Therefore a grower can control active growth towards harvest by reducing amount of nitrogen. Also, the use plant growth regulators may be essential to manipulate aspects related to senescence and colour development.

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4. PAPER 1: THE EFFECT OF HAND THINNING ON FRUIT SIZE AND YIELD OF 'NULES CLEMENTINE' MANDARIN (*Citrus reticulata* Blanco)

Abstract

Fruit size is an important aspect in citriculture and plays a role in fruit acceptance by consumers whose preference is for larger fruit. Hand thinning was conducted for three consecutive years to enhance fruit size. Thinning was carried out on 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) either early (mid or late January) or late (mid or late February) with either light, medium or heavy thinning severity being applied. In the first year, both fruit size and yield were not affected by early-medium or late-light hand thinning treatments. In the second year (only light thinning severity applied), the fruit of marketable size category (≥55 mm diameter) was significantly increased by both early-light (12% fruitlets removed) and late-light (27% fruitlets removed) thinning treatments, although there was no significant difference on fruit size and yield compared to other thinning treatments. During the third year (light, medium and heavy thinning severity applied), fruit of marketable size was increased by the late-heavy (60% fruitlets removed) thinning treatment, but with a significant reduction in yield by 18.8 kg/tree (37%) compared to the unthinned control. Although only fruit size was evaluated, improved external fruit quality or reduced cull at packout was expected where trees were thinned, since blemished fruit were also removed at the time of thinning.

Introduction

Fruit size is an important external fruit quality factor in fresh citrus production, and plays a role in determining fruit acceptance by consumers (Guardiola, Garcia-Mari & Agusti, 1984). The possibilities of improving fruit size in citrus rest on either genetic improvement, crop load management or modification of cultural practices (Vanniere & Arcuset, 1992).

Fruit size increases with the number of leaves available to supply carbohydrates to the growing fruit (Gilfillan, 1990). Hence, intensive pruning (Oren, 1988) and high concentration of ethephon (Ethrel®) which promote leaf abscission (Young *et al.*, 1970) had variable results on yield and fruit size. Therefore, thinning fruit in alternate bearing cultivars, which tend to produce small fruit in heavy crop years, can help to improve fruit size of the remaining fruit by reducing intersink competition for photosynthates (Guardiola, Almela & Barres, 1988), and maintain constant crop production (Harty & Sutton, 1992; Moss, 1972). Methods of increasing fruit size in citrus including thinning have been reviewed by Gilfillan (1987) and more recently by Guardiola and Garcia-Luis (2000).

The effect of reducing the number of fruitlets by thinning using ethephon after physiological fruit drop was an increase in fruit size of 'Valencia' and 'Washington Navel' oranges [*C. sinensis* (L). Osbeck] (Hield, Burns & Coggins, 1962; Hutton, 1992; Stewart, Hield & Brannaman, 1952). However, the problem with chemical thinning, aside from leaf abscission, is that the success of chemical thinning is negatively affected by weather conditions such as rain after application (Gilfillan, 1987). Alternatively, hand thinning increased fruit size of 'Valencia' and 'Tomango' oranges when 30% of the fruit was removed (Gilfillan, 1987; Rabe, 1991), 'Clausellina Satsuma' mandarin (*C. unshui* Marc.) (Zaragoza *et al.*, 1992) and 'Satsuma' mandarin (Harty & Sutton, 1992; Sutton & Harty, 1990). Consequently, hand thinning results vary from 10 to 14% yield reduction (Rabe, 1991) whilst ethephon resulted in 23% reduction of total yield (Gilfillan, 1987). Hand thinning requires no chemical formulation to improve fruit size and therefore it could be an important tool in organic agriculture and consumer satisfaction if its results were reliable.

In South Africa, the exact timing for hand thinning has not been established or intensively studied. The aim of this study was to determine the effect of hand thinning carried out in mid January and mid February (4 to 6 weeks after physiological fruit drop) on fruit size and yield of 'Nules Clementine' mandarin.

Materials and Methods

Plant material and site selection. During the first year (1998), this experiment was conducted at Welgevallen Experimental Farm, Stellenbosch, South Africa (34°S, 19°E, winter rainfall area, altitude ca. 119 m) on seven-year-old 'Nules Clementine' mandarin trees budded onto 'Troyer' citrange [*Poncirus trifoliata* (L.) Raf. x *C. sinensis*] rootstock. For the second and third years (1999 and 2000), the experiment was conducted at Goedvertrouw, a commercial farm, near Stellenbosch, South Africa (34°S, 19°E, winter rainfall area, altitude ca. 100 m) on six-year-old 'Nules Clementine' mandarin trees budded onto 'Carrizo' citrange rootstock. The trees used for the study were selected for uniformity of tree size and health.

Treatments and statistical design. Thinning was carried out either early (mid or late January) or late (mid or late February) with either light, medium or heavy thinning severity being applied as follows:

	Early			Late		
	Light	Medium	Heavy	Light	Medium	Heavy
1998		X		X		
1999	X			X		
2000	X	X			X	X

There was also an unthinned control treatment in each year. To determine which fruit to remove at each thinning date, ≈100 fruit were harvested and their fruit diameter measured, from which the smallest ≈25% (light thinning severity) or ≈50% (heavy thinning severity) of the fruit, including the blemished fruit were removed. The number of fruit removed per tree was recorded. Actual thinning percentage was determined by dividing the number of fruit removed by the sum of the number of fruit removed and the number of fruit harvested per tree. The number of fruit harvested per tree was determined from the fruit yield and average fruit weight data. All experiments were arranged in a randomized complete block design with three to six treatments and 10 to 15 single-tree replicates.

Fruit size and yield. At harvest, fruit size and total yield were determined. Fruit size was determined by randomly selecting a sample of ≈7 kg of fruit and separating the fruit into size categories at a commercial packinghouse on an electronic grader. The commercial size categories and corresponding fruit diameters were:

Size category	Fruit diameter range (mm)
1X	≥ 68-72
1	64-68
2	59-64
3	55-59
4	51-55
5	48-51
Undersize (U/S)	≤ 48

Yield was determined as total fruit weight.

Statistical analysis. The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyze the fruit size and yield data (SAS Institute Inc., 1990).

Results

Yield. In 1998 and 1999, yield was not significantly affected by thinning treatments (Tables 1 and 2). However, in 2000 the late-heavy (60% fruit removed) thinning treatment reduced yield by 37% (18.8 kg/tree) compared to the unthinned control (Table 3). These data show no clear trend in terms of thinning severity, but, on average, the heavy thinning treatments reduced yield.

[Tables 1, 2 and 3]

Fruit size. In 1998, no significant difference in fruit size distribution was obtained by thinning treatments (Table 1). During 1999, although there was no significant differences in marketable fruit category (MFC) (≥55 mm diameter), the early-light (12% fruitlets removed) and late-light (27% fruitlets removed) thinning treatments tended to have a higher amount of fruit ≥55 mm in diameter than the unthinned control (Table 2). In 2000, medium and heavy severity hand thinning resulted in a higher proportion of calibre 1 to 3 fruit than unthinned control trees and a lower proportion of calibre 4 and smaller (Table 3). However, there was no significant difference in MFC among thinning treatments.

[Tables 1, 2 and 3]

Discussion and Conclusion

Over the 3 years that the hand thinning study was conducted on 'Nules Clementine' mandarin, there was no consistent treatment effect of thinning severity or timing on fruit size or yield. However, early-light thinning in 1999 resulted in a higher proportion of larger fruit without adversely affecting total yield. Whereas, heavy thinning in 2000 resulted in a higher proportion of larger fruit and total yield was adversely affected. Gilfillan (1990) recommended light thinning (20 to 30% fruitlets removed) in 'Valencia' orange for beneficial results on fruit size. Harty and Sutton (1992) obtained seemingly opposite results with 'Silverhill Satsuma' mandarin. However, in the literature it is speculated that thinning must be severe for the effect of reduction in competition to be evident (Zaragoza *et al.*, 1992). Hilgeman, True and Dunlap (1964), Rabe (1991) and Zaragoza *et al.* (1992) found that there was a good relationship between the severity of thinning and fruit size improvement for 'Kinnow' mandarin, 'Tomango' and 'Valencia' oranges, and 'Clausellina Satsuma' mandarin, respectively. Heavy thinning results in a leaf to fruit ratio of 1:12 in 'Satsuma' mandarin, and therefore there are more photosynthates available for fruit development (Sutton & Harty, 1990). However, before hand thinning of fruit can be considered for improving fruit size it should be determined that the only factor which is limiting fruit size is excessive fruit load and not some other deficiencies (Rabe, 1991).

Results obtained in this study reflect no clear effect of timing of thinning on fruit size enhancement and yield. In previous research (Gilfillan, 1990; Sutton & Harty, 1992), hand thinning just after physiological fruit drop, i.e. November/December in the southern hemisphere, at the beginning of the cell enlargement stage of fruit development (Bain, 1958), had a favourable effect on fruit size enhancement. Also the sink strength of fruitlets, which determines fruitlet growth, is largely determined during the early stage of fruit development (Guardiola & Garcia-Luis, 1997). The lack of effect of timing of hand thinning in this study could be due to thinning being done 4 to 6 weeks after the physiological fruit drop period, i.e. too late to have the desired effect.

Although the commercial packout percentage was not quantified in this study, a higher packout is expected since blemished fruit were removed when hand thinning was applied. Furthermore, hand thinning could be worthwhile to reduce alternate bearing cycles. In conclusion, due to hand thinning being conducted relatively late no consistent treatment effect on fruit size or yield could be demonstrated.

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Table 1. Effect of hand thinning after physiological fruit drop on fruit size and yield of 'Nules Clementine' mandarin at Welgevallen Experimental Farm, Stellenbosch (1998).

Treatments	Thinning date	Fruit removed per tree	Fruit size per category by mass (%)				Total yield (kg/tree)
			≥ 1X[z] ≥ 68mm[y]	1–3 55–68mm	4–5 48–55mm	U/S ≤48mm	
Unthinned control		-	0.0 a[x]	23.6 a	70.7 a	5.7 a	44.9 a
Early-medium (≤ 30mm + blemished)	30 Jan	339	0.3 a	30.1 a	64.7 a	4.9 a	37.4 a
Late-light (≤ 35mm + blemished)	16 Feb	224	0.0 a	22.8 a	72.4 a	4.8 a	42.3 a
LSD (P≤ 0.05)			0.5	14.2	13.4	3.8	9.6

[z] Calibre used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 2. Effect of hand thinning after physiological fruit drop on fruit size and yield of 'Nules Clementine' mandarin at Goedvertrouw, Stellenbosch (1999).

Treatments	Thinning date	Actual thinning (%)	Fruit removed per tree	Fruit remained per tree	Fruit size per category by mass (%)				Total yield	Actual kg
					≥1X[z] ≥68mm[y]	1–2 59–68mm	3–5 48–55mm	< 3–U/S < 55mm	(kg/tree)	>55 mm
Unthinned control		-	-	786	7.9 bc[x]	28.2 b	27.5 a	36.4 a	48.5 a	30.6 b
Early-light (≤23mm + blemished)	20 Jan	12	107	714	16.9 a	40.8 ab	23.3 a	19.0 bc	51.9 a	41.5 a
Early-light (≤24mm+blemished)	20 Jan	21	164	604	16.1 a	45.1 a	21.3 a	17.5 c	45.6 a	36.9 ab
Early-light (≤25mm + blemished)	20 Jan	28	295	765	8.4 abc	35.9 ab	27.1 a	28.6 abc	47.1 a	33.4 ab
Late-light (≤30mm + blemished)	15 Feb	23	219	736	3.2 c	28.8 b	27.4 a	30.5 ab	46.8 a	33.8 ab
Late-light (≤32mm + blemished)	15 Feb	27	273	725	8.4 abc	42.8 a	28.7 a	20.1 bc	51.1 a	40.4 a
LSD (P≤ 0.05)					8.8	13.0	8.3	12.4	11.8	9.8
Sign. Level					0.0217	0.0425	0.4330	0.0185	0.8486	0.1771

[z] Calibre used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 3. Effect of hand thinning after physiological fruit drop on fruit size and yield of 'Nules Clementine' mandarin at Goedvertrouw, Stellenbosch (2000).

Treatments	Thinning date	Actual thinning (%)	Fruit removed per tree	Fruit remained per tree	Fruit per size category by mass (%)				Total yield (kg/tree)	Actual kg >55mm
					≥1X[z] ≥68mm[y]	1–3 55–68mm	4–5 48–55mm	U/S ≤48mm		
Unthinned control		-	-	543	0.4 a[x]	55.9 c	40.9 a	2.7 a	50.6 a	31.7 ab
Early-light (≤ 26mm + blemished)	19 Jan	26	203	574	1.4 a	64.3 bc	33.3 ab	1.0 b	48.9 a	34.5 a
Early-medium (≤ 28mm + blemished)	19 Jan	42	321	441	3.9 a	72.7 ab	22.8 bc	0.6 b	41.4 ab	28.0 ab
Late-medium (≤ 36mm + blemished)	15 Feb	45	348	421	1.4 a	72.6 ab	25.3 bc	0.7 b	37.6 ab	28.8 ab
Late-heavy (≤ 38mm + blemished)	15 Feb	60	487	330	4.6 a	77.7 a	16.8 c	0.9 b	31.8 b	25.4 b
LSD (P≤ 0.05)					4.3	9.9	10.6	1.3	13.6	8.8
Sign. Level					0.2368	0.0007	0.0005	0.0106	0.0446	0.2690

[z] Calibre used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

5. PAPER 2: THE EFFECT OF MULTIPLE DICHLORPROP SPRAYS WITH DIFFERENT SURFACTANTS ON FRUIT SIZE AND YIELD OF 'NULES CLEMENTINE' MANDARIN (*Citrus reticulata* Blanco)

Abstract

Clementine mandarin (*Citrus reticulata* Blanco) and other mandarin selections are prone to producing small fruit. The use of a plant growth regulator, dichlorprop, had been reported to improve fruit size without any thinning effect. In this study, two sets of trials were done, namely dichlorprop multiple sprays and dichlorprop with different surfactants at different concentrations, just after the physiological drop period. It was evident that dichlorprop increase fruit size through accelerating fruit growth. However, it was observed that higher concentrations (100, 150, 200 mg. L^{-1}) led to nonsignificant thinning. Multiple sprays did not result in any enhanced fruit size or reduced yield or adverse internal fruit quality. Dichlorprop and different surfactants significantly improved fruit size without yield reduction. The best treatment was the combination of dichlorprop (100 mg. L^{-1}) with Orchex (150 mL. 100 L^{-1}) which significantly improved fruit size (>55 mm diameter) by 17 kg (35%) compared to the unsprayed control. A yield reduction of up to 35% was experienced in the second year; however, fruit size was not affected. Dichlorprop in combination with different surfactant did not show any effect on internal fruit quality. However, juice percentage tended to be reduced by some dichlorprop applications during the second year.

Introduction

Fruit size in citrus is the determining factor for fruit acceptance by consumers. Premiums are paid for larger fruit, and increasing fruit size greatly increases returns to the grower. Small fruit size is a problem in 'Clementine' mandarin selections that are genetically prone to bear small fruit (Rabe, Koch & Theron, 1995; Vanniere & Arcuset, 1992). Gilfillan (1987) reviewed the factors influencing the final fruit size in oranges and practical measures to fruit size improvement.

Synthetic auxins have been applied to increase final fruit size by enhancing fruit growth without reducing yield, even though yield reduction depends on the nature of auxin (Guardiola, Almela & Barres, 1988), and the time of application and concentration applied (Agusti, Almela & Aznar, 1992; Ortola, Monerri & Guardiola, 1991). Naphthalene acetic acid (NAA) had been successfully used to increase fruit size in mandarins (Ortola, Monerri & Guardiola, 1997; Wheaton, 1981), 'Star Ruby' grapefruit (Greenberg, Hertzano & Eshel, 1992), but its success is mainly owing to its thinning effect. The thinning effect caused by NAA negates the benefits of improving fruit size by reducing total yield.

Application of the butylglycol ester of 2,4 dichlorophenoxy propionic acid (dichlorprop) after the physiological fruit drop period, increased final fruit size of 'Clementine' mandarin (Agusti *et al.*, 1992; Rabe *et al.*, 1995; Vanniere & Arcuset, 1992), 'Satsuma' mandarin (Agusti *et al.*, 1994), and 'Fortune' mandarin (El-Otmani *et al.*, 1993). The thinning effect of dichlorprop is most marked when it is applied shortly after the physiological fruit drop period (Agusti *et al.*, 1992), when the average fruit size is 20 mm diameter for 'Clementine' mandarin (Agusti *et al.*, 1994).

This paper reports on the effect of dichlorprop multiple sprays, and when applied with different spreaders on fruit retention and growth, fruit size, yield, and internal quality of 'Nules Clementine' mandarin after the physiological fruit drop period.

Materials and Methods

Experiment 1. Dichlorprop multiple sprays

Plant materials and site selection. The study was conducted at two sites in the Western Cape region of South Africa. (1) Welgevallen Experimental Farm, Stellenbosch, (34°S; 19°E, winter rainfall area, altitude ca. 119 m), on nine-year-old 'Nules Clementine' mandarin trees budded onto 'Troyer' citrange [*Poncirus trifoliata* (L.) Raf. x *C. sinensis*] rootstock and (2) Grabouw (34°S, 19°E, winter rainfall area, altitude ca. 305 m) on 'Nules Clementine' mandarin trees planted in 1993, budded onto 'Carrizo' citrange. Trees were selected for health and uniformity of tree size.

Treatments and statistical design. After the physiological fruit drop (APFD) period, multiple dichlorprop sprays were applied by handguns as full cover foliar sprays. There was also an unsprayed control. At Welgevallen, the first spray was on 13/12/1999 (fruit diameter: 13 mm), second spray 14 days APFD and third spray was 28 days APFD. The treatments are outlined in Table 1. At Grabouw, the trial block had already been sprayed once. Thus, the first spray was on 09/12/1999 (estimated fruit diameter: 11mm), second spray was 14 days APFD and third spray was 28 days APFD. The treatments are outlined in Table 3. The trials were laid out as randomized complete block designs with six treatments at Welgevallen (10 single-tree replicates), and three treatments at Grabouw (10 single-tree replicates).

Experiment 2. Dichlorprop with different surfactants

Plant materials and site selection. The study was conducted on two sites in the Western Cape region of South Africa for two consecutive years. In 1999, the study was conducted at Goedvertrouw, a commercial farm, near Stellenbosch, (Site A) (34ºS; 19ºE, winter rainfall area, altitude ca. 100 m), and Grabouw (Site B), (34°S, 19°E, winter rainfall area, altitude ca. 305 m). At both sites, trees that were used was 'Nules Clementine' mandarin, planted in 1993 budded onto 'Carrizo' citrange. Trees were selected for health and uniformity of tree size.

Treatments and statistical design. Trees were sprayed with dichlorprop with different surfactants just after physiological fruit drop period, by handguns as full cover foliar sprays when the average fruit size was 7 mm (01/12/1999) and 9 mm (06/12/2000), at the two sites, respectively. The trials were laid out as randomized complete block designs with ten treatments (10 single-tree replicates) at both sites. In year 2000, treatments were buffered using Commodobuff (see footnote in Table 7). The treatments for both sites are outlined in Tables 5 and 7, respectively.

Fruit retention and growth measurements. During the first year, one twig of the previous season's flush, 20 cm in length with approximately 20 fruit was tagged per tree, for fruit retention measurements. Fruitlets were counted at the spray date and every 2 weeks thereafter until mid January. For fruit growth, 50 fruitlets per treatment were tagged at the spray date using replicates 1 to 5 (10 fruits per replicate, i.e. 5 on each side of the tree), and fruit growth was measured every 2 weeks until harvest.

Fruit size and yield determination. At harvest, fruit size and total yield were determined. Fruit size was determined by randomly selecting a sample of 15 to 20% of the yield, and separating the fruit into size categories at a commercial packinghouse on an electronic grader. Yield was measured as total fruit weight.

Internal fruit quality. At harvest 12 fruit of the same size (six from each side of the tree) for each replicate were collected for internal fruit quality analysis. Fruit were squeezed using a hand reamer (citrus juicer). Juice was strained through two layers of Muslin cloth and juice content (%) was determined by subtracting the mass of the reamed peel from the total fruit mass. Total soluble solids (TSS) of the juice were determined by using an Atago hand refractometer; titratable acid (TA) was determined by titrating against 0.1 N NaOH using phenolphthalein indicator and the TSS:TA was calculated.

Statistical analysis. The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyse the fruit size, yield and internal fruit quality data (SAS Institute Inc., 1990).

Results and Discussion

Fruit growth. Dichlorprop application increased the normal fruit growth pattern at Welgevallen, but there was no difference between single and multiple sprays (Figure 1). At Grabouw there was a slight difference in fruit growth between the treatments (Figure 2). Also, at Goedvertrouw, the growth was greatly increased when dichlorprop (50 mg. L^{-1}) was sprayed with Orchex (250 mL. 100 L^{-1}), throughout the fruit development as compared to the unsprayed control (Figure 3). Increase in fruit growth can be seen from two weeks after the dichlorprop spray application. Koch, Theron and Rabe (1996) indicated that small fruit normally remained smaller up to harvest as compared to the large fruit. Therefore, dichlorprop stimulate fruit growth directly as described by (Guardiola *et al.*, 1988) to bring a change in the normal growth pattern and thus achieving larger fruit at harvest.

[Figures 1, 2 and 3]

Fruit drop. Single sprays retained fruit better than multiple sprays which accelerated the normal fruit drop pattern at both Welgevallen and Grabouw (Figures 4 and 5). Vanniere and Arcuset (1992) observed dichlorpop induces thinning with early spray application and at higher concentration. Also, Koch *et al.* (1996) observed that fruit retention percentage was lowered by higher dichlorprop concentrations. However, dichlorprop with different surfactants at Goedvertrouw showed no effect on accelerating the normal fruit drop pattern (Figure 6). Therefore, dichlorprop does not thin fruit as the mode of its effectiveness, but thinning could be considered as a secondary effect (Agusti *et al.*, 1992).

[Figures 4, 5 and 6]

Yield. Multiple dichlorprop sprays did not show any significant difference in yield at the two sites, i.e. Welgevallen and Grabouw (Tables 1 and 3). Also, dichlorprop with different surfactants resulted in no effect on yield at Goedvertouw (Table 5). At Grabouw in 2001, however, dichlorprop with different surfactants significantly reduced yield (Table 7). The most remarkable reduction of ≈20 kg (≈35%) was observed when dichlorprop (100 mg. L^{-1}) was applied with Orchex (200 mL. 100 L^{-1}). Yield reduction less than 10% was also experienced with earlier applications (Rabe *et al.*, 1995). Reduction in yield caused by dichlorprop could be a benefit in reducing harvest costs if large fruit is guaranteed.

[Tables 1, 3 and 5]

Fruit size. Although fruit size of individual fruit size categories was not significantly affected by dichlorprop multiple sprays (Table 1), the amount of fruit >55mm was significantly improved by one or more dichlorprop sprays at 50 mg. L^{-1} than the unsprayed control. However, there were no benefits gained with increasing number of sprays. Also, at Grabouw, fruit size was not substantially affected by dichlorprop multiple sprays at 100 mg. L^{-1} (Table 3). Therefore, one spray is sufficient to achieve the desired results.

Dichlorprop with different surfactants significantly enhanced fruit size (P=0.01) at Goedvertouw (Table 5). Dichlorprop reduced the proportion of small fruit (48-55 mm) at 50 mg. L^{-1} and consequently increased large fruit (>55 mm). However, the only significant difference observed among the dichlorprop treatments was when dichlorprop (100 mg. L^{-1}) was applied with Orchex (150 mL. 100 L^{-1}). At Grabouw (2001), fruit size (>55 mm) was significantly increased by a combination of dichlorprop (100 mg. L^{-1}) with Agral 90 (20 mL. 100 L^{-1}), dichlorprop (50 mg. L^{-1}) with Orchex (200 mL. 100 L^{-1}) and dichlorprop (50 mg. L^{-1}) with Break-thru (50 mL. 100 L^{-1}) (Table 7). The marginal difference produced by the rest of treatments was due to the yield reduction experienced. Buffered treatments using Commodobuff in 2000 resulted in no effect on fruit size when compared to the unbuffered control of the same concentration of dichlorprop and surfactants (Table 7).

[Tables 1,3, 5 and 7]

Internal fruit quality. Dichlorprop multiple sprays did not have any significant effect on any of the internal fruit quality variables measured at both Welgevallen and Grabouw (Tables 2, 4 and 6). However, at Goedvertrouw, dichlorprop (100 mg. L^{-1}) with Orchex (250 mL. 100 L^{-1}) significantly reduced the juice percentage. Miller, Bird and Maritz (1999) reported that reduction in juice percentage following the dichlorprop application was due to granulation experienced with larger fruit.

[Tables 2, 4 and 6]

In conclusion, dichlorprop at higher concentrations than 100 mg. L^{-1} might lead to severe thinning. However, where thinning was observed, yield was not necessarily affected. Fruit size was significantly improved by dichlorprop from 50 to 100 mg. L^{-1} without yield reduction. Orchex had an optimum response at 150 mL. 100 L^{-1}. BP Cipron and Orchex seemed to give better results than Agral 90. Break-thru seemed to be more effective in lower dichlorprop concentrations. The effect of surfactants needs further investigation. Internal fruit quality was not affected by multiple dichlorprop sprays and dichlorprop with different surfactants.

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Table 1. Effect of multiple dichlorprop sprays on fruit size and yield of 'Nules Clementine' mandarin at Welgevallen, Stellenbosch (2000). First spray was on 13/12/1999 (fruit diameter: 13 mm), second spray was 14 days APFD (fruit diameter: 20 mm) and third spray was 28 days APFD (fruit diameter: 27 mm).

Treatments	Dichlorprop Conc. (mg. L^{-1})	Fruit size category by mass (%)				Total yield (kg/tree)	Actual kg >55 mm
		≥1X[z] ≥68 mm[y]	1-3 55-68 mm	4-5 48-55 mm	U/S <48 mm		
Control (Unsprayed)		1.3 b[x]	61.6 b	35.2 a	2.0 a	50.7 ab	31.9 b
1 spray	50	5.1 ab	65.0 ab	25.2 ab	1.7 a	57.8 a	40.5 a
2 sprays	50	4.1 ab	75.1 a	19.5 b	1.3 a	51.1 ab	40.5 a
3 sprays	50	3.1 b	74.4 a	21.5 b	1.0 a	51.8 ab	40.2 a
1 spray	100	7.7 a	70.7 ab	20.4 b	0.8 a	46.8 b	36.7 ab
2 sprays	100	2.7 b	72.1 ab	24.1 b	1.1 a	47.5 b	35.5 ab
LSD (P=0.05)		4.5	11.8	11.0	1.5	9.3	6.2
Sign. Level		0.0935	0.1494	0.0681	0.5892	0.2256	0.041

[z] Calibre used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 2. Effect of multiple dichlorprop sprays on internal fruit quality of 'Nules Clementine' mandarin at Welgevallen, Stellenbosch (2000). First spray was on 13/12/1999 (fruit diameter: 13 mm), second spray was 14 days APFD (fruit diameter: 20 mm) and third spray was 28 days APFD (fruit diameter: 27 mm).

Treatments	Dichlorprop Conc. (mg. L^{-1})	Internal fruit quality			
		Juice %	TSS	TA	TSS:TA Ratio
Control		53.00 a[z]	11.74 ab	1.16 a	10.16 a
1 spray	50	52.14 a	11.36 b	1.13 a	10.08 a
2 sprays	50	56.36 a	11.72 ab	1.14 a	10.22 a
3 sprays	50	52.58 a	11.74 ab	1.16 a	10.12 a
1 spray	100	54.14 a	11.92 a	1.17 a	10.28 a
2 sprays	100	53.18 a	11.86 a	1.17 a	10.12 a
LSD (P=0.05)		6.7	0.5	0.1	0.7
Sign. Level		0.8038	0.2048	0.9206	0.9927

[z] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 3. Effect of multiple dichlorprop sprays (100 mg. L^{-1}) on fruit size and yield of 'Nules Clementine' mandarin at Grabouw (2000). First spray was on 09/12/1999 (estimated fruit diameter: 11 mm), second spray was 14 days APFD (fruit diameter: 16 mm) and third spray was 28 days APFD (fruit diameter: 22 mm).

Treatments*	Fruit size category by mass (%)				Total yield	Actual kg
	≥1X[z]	1-3	4-5	U/S	(kg/tree)	>55mm
	≥68 mm[y]	55-68 mm	48-55 mm	<48 mm		
1 spray	1.1 b[x]	58.6 b	29.6 a	0.7 a	46.0 a	27.5 b
2 sprays	3.2 a	78.8 a	17.9 a	0.1 a	43.9 a	35.7 a
3 sprays	0.8 b	67.5 ab	31.2 a	0.5 a	48.5 a	32.8 ab
LDS (P=0.05)	2.0	19.1	15.4	0.6	6.0	7.7
Sign. Level	0.0391	0.1135	0.1672	0.1365	0.2930	0.1040

[z] Caliber used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

* At the time of initiating the trial, one spray was already applied.

Table 4. Effect of multiple dichlorprop sprays (100 mg. L^{-1}) on internal fruit quality of 'Nules Clementine' mandarin at Grabouw (2000). First spray was on 09/12/1999 (estimated fruit diameter: 11 mm), second spray was 14 days APFD (fruit diameter: 16 mm) and third spray was 28 days APFD (fruit diameter: 22 mm).

Treatments*	Internal fruit quality			
	Juice %	TSS	TA	TSS:TA Ratio
1 spray	52.92 a[z]	11.58 a	0.98 a	11.8 a
2 sprays	49.10 a	11.66 a	0.96 a	12.1 a
3 sprays	52.58 a	11.98 a	1.02 a	11.84 a
LDS (P=0.05)	5.9	0.7	0.1	1.4
Sign. Level	0.3057	0.4549	0.5685	0.8725

[z] Numbers in columns with different letters are significantly different ($P \leq 0.05$, LSD).

* At the time of initiating the trial, one spray was already applied.

Table 5. Effect of dichlorprop in combination with different surfactants on fruit size and yield of 'Nules Clementine' mandarin at Goedvertrouw, Stellenbosch (2000). Trees were sprayed on 24/11/1999 (fruit diameter: 7 mm).

Treatments	Dichlorprop Conc. (mg. L^{-1})	Surfactant Conc. (mL. 100 L^{-1})	Fruit size category by mass (%)				Total yield (kg/tree)	Actual kg >55 mm
			≥1X[z] ≥68 mm[y]	1-3 55-68 mm	4-5 48-55 mm	U/S <48 mm		
Control (Unsprayed)			2.0 de[x]	61.0 b	36.1 a	0.7 a	49.9 a	31.4 c
Dichlorprop, Orchex	25	250	3.3 de	72.0 ab	24.3 ab	0.4 a	58.4 a	44.0 ab
Dichlorprop, Orchex	50	250	8.8 bcde	74.5 a	15.4 bc	1.3 a	49.6 a	41.4 ab
Dichlorprop, Orchex	100	250	20.9 a	66.2 ab	12.1 bc	0.8 a	49.7 a	43.3 ab
Dichlorprop, Orchex	100	150	18.0 ab	71.5 ab	10.56 c	0.5 a	54.2 a	48.5 a
Dichlorprop, BP Cipron	50	250	11.4 abcd	72.3 ab	15.9 bc	0.4 a	49.7 a	41.6 ab
Dichlorprop, Agral 90	50	20	14.0 abc	69.1 ab	19.9 bc	1.3 a	47.2 a	39.2 b
Dichlorprop, Agral 90	100	20	4.5 cde	69.7 ab	24.6 ab	1.2 a	55.4 a	41.1 ab
Dichlorprop, Break-thru	50	50	1.7 e	75.1 a	22.0 bc	1.2 a	55.4 a	42.5 ab
Dichlorprop, Break-thru	100	50	6.3 cde	75.5 a	17.1 bc	1.1 a	51.6 a	42.2 ab
LSD (P=0.05)			9.7	11.6	13.0	1.4	12.4	7.6
Sign. Level			0.0004	0.3289	0.0093	0.8183	0.7416	0.0142

[z] Caliber used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 6. Effect of dichlorprop in combination with different surfactants on internal fruit quality of 'Nules Clementine' mandarin at Geodvertrouw, Stellenbosch (2000). Trees were sprayed on 24/11/1999 (fruit diameter: 7 mm).

Treatments	Dichlorprop Conc. (mg. L^{-1})	Surfactants Conc. (mL. 100 L^{-1})	Internal fruit quality			
			Juice %	TSS	TA	TSS:TA Ratio
Control (Unsprayed)			52.28 a[z]	11.10 a	1.16 ab	9.62 ab
Dichlorprop, Orchex	25	250	49.26 ab	11.28 a	1.18 ab	9.54 ab
Dichlorprop, Orchex	50	250	51.00 a	11.38 a	1.16 ab	9.78 ab
Dichlorprop, Orchex	100	250	44.76 b	11.42 a	1.18 ab	9.66 ab
Dichlorprop, Orchex	100	150	46.82 ab	10.86 a	1.09 b	9.94 a
Dichlorprop, BP Cipron	50	250	47.00 ab	11.14 a	1.12 ab	9.96 a
Dichlorprop, Agral 90	50	20	49.80 ab	10.98 a	1.14 ab	9.62 ab
Dichlorprop, Agral 90	100	20	50.46 ab	11.74 a	1.18 ab	9.88 a
Dichlorprop, Break-thru	50	50	50.08 ab	11.04 a	1.19 b	9.22 b
Dichlorprop, Break-thru	100	50	48.84 ab	11.44 a	1.18 ab	9.74 ab
LSD (P=0.05)			5.8	0.9	0.1	0.7
Sign. Level			0.3213	0.7399	0.4285	0.5178

[z]Numbers in columns with different letters are significantly different ($P \leq 0.05$, LSD).

Table 7. Effect of dichlorprop with different surfactants on fruit size and yield of 'Nules Clementine' mandarin at Grabouw (2001). Trees were sprayed on 06/12/2000 (fruit diameter: 9 mm).

Treatments	Dichlorprop Conc. (mg. L^{-1})	Surfactants Conc. (mL. 100 L^{-1})	Fruit size category by mass (%)				Total yield (kg/tree)	Actual kg >55mm
			>1X [z]	1-3	4-5	U/S		
			>68mm [y]	55-68mm	48-55mm	<48mm		
Control (Unsprayed)	-	-	0.0 a[z]	29.2 d	64.9 a	6.0 a	58.9 a	16.2 bc
Dichlorprop, Agral 90	25	20	0.2 a	33.1 cd	63.5 ab	3.1 ab	46.9 dc	15.1 c
Dichlorprop, Agral 90	50	20	0.0 a	41.8 bcd	57.7 abc	0.6 b	48.5 bc	18.6 abc
*Dichlorprop, Agral 90	100	20	1.5 a	47.0 abc	50.7 abcd	0.9 b	56.9 ab	27.4 a
Dichlorprop, Orchex	25	200	0.2 a	47.4 abc	52.2 abcd	0.2 b	49.1 bc	20.9 abc
Dichlorprop, Orchex	50	200	0.6 a	56.4 ab	42.2 cd	0.8 b	50.6 abc	26.9 a
Dichlorprop, Orchex	100	200	1.8 a	61.2 a	35.8 d	1.2 b	38.5 d	25.5 ab
Dichlorprop, Orchex	50	100	0.3 a	53.5 ab	46.0 bcd	0.1 b	47.7 bcd	23.3 abc
Dichlorprop, Break-thru	50	50	1.2 a	51.2 ab	47.5 abcd	0.1 b	49.5 abc	26.9 a
**Dichlorprop, Agral 90	100	20	1.6 a	48.2 abc	49.4 abcd	0.1 b	48.3 bcd	21.4 abc
LSD (P= 0.05)			2.0	17.8	18.6	3.3	9.8	10.0
Sign. level			0.3721	0.0076	0.028	0.5869	0.0001	0.1330
CONTRAST: (Pr>F)			**>1X**	**1-3**	**4-5**	**U/S**	**Actual**	
Buffered vs. Not buffered			0.8678	0.6416	0.6641	0.9645	0.2335	

* Treatment buffered with CommandoBuff ** Not buffered.

[z] Calibre used in commerce: U/S = undersized.

[y] Mean fruit diameter per category.

[x] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

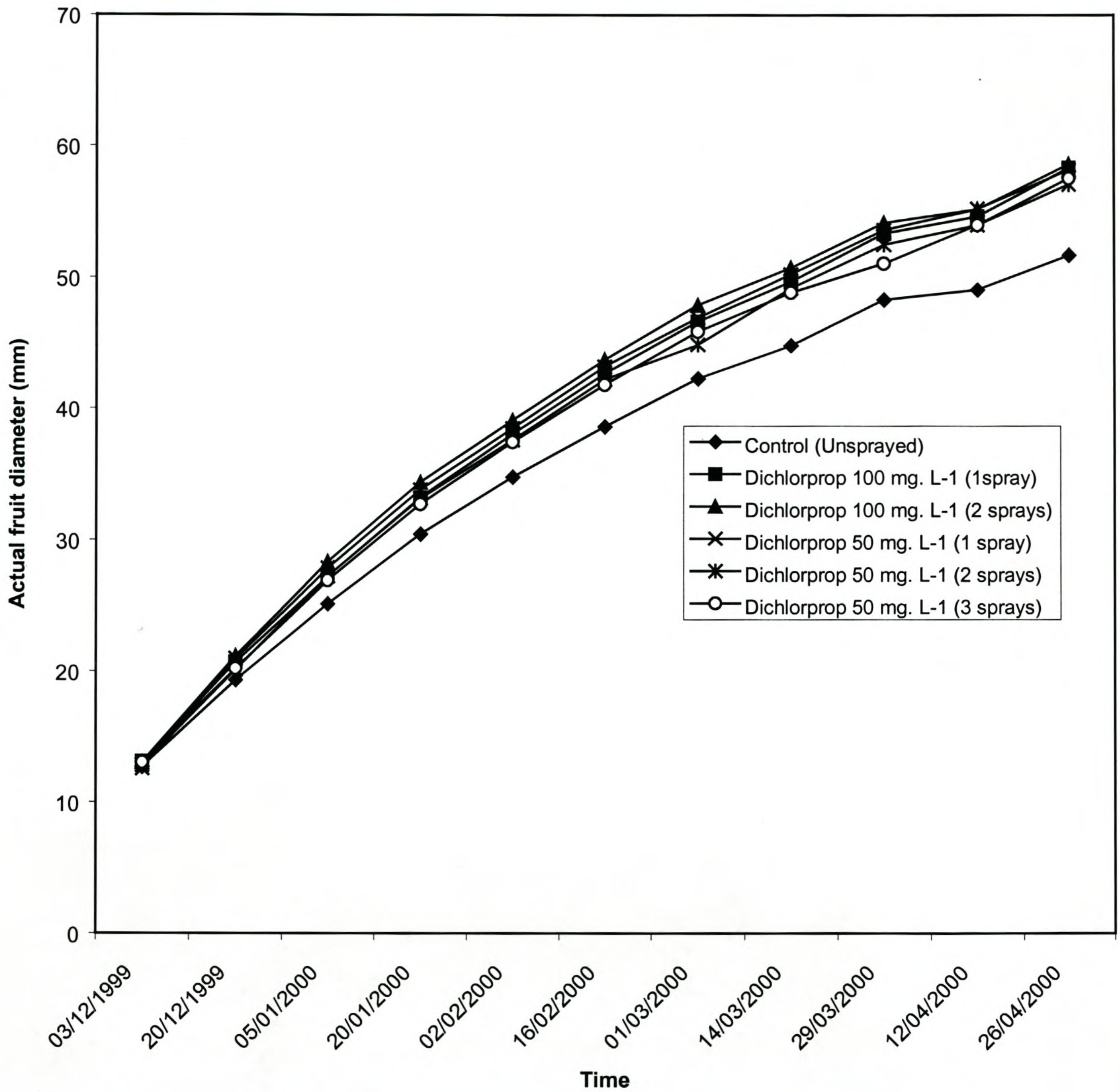

Figure 1. Fruit growth curves of 'Nules Clementine' mandarin sprayed with multiple dichlorprop sprays at Welgevallen, Stellenbosch.

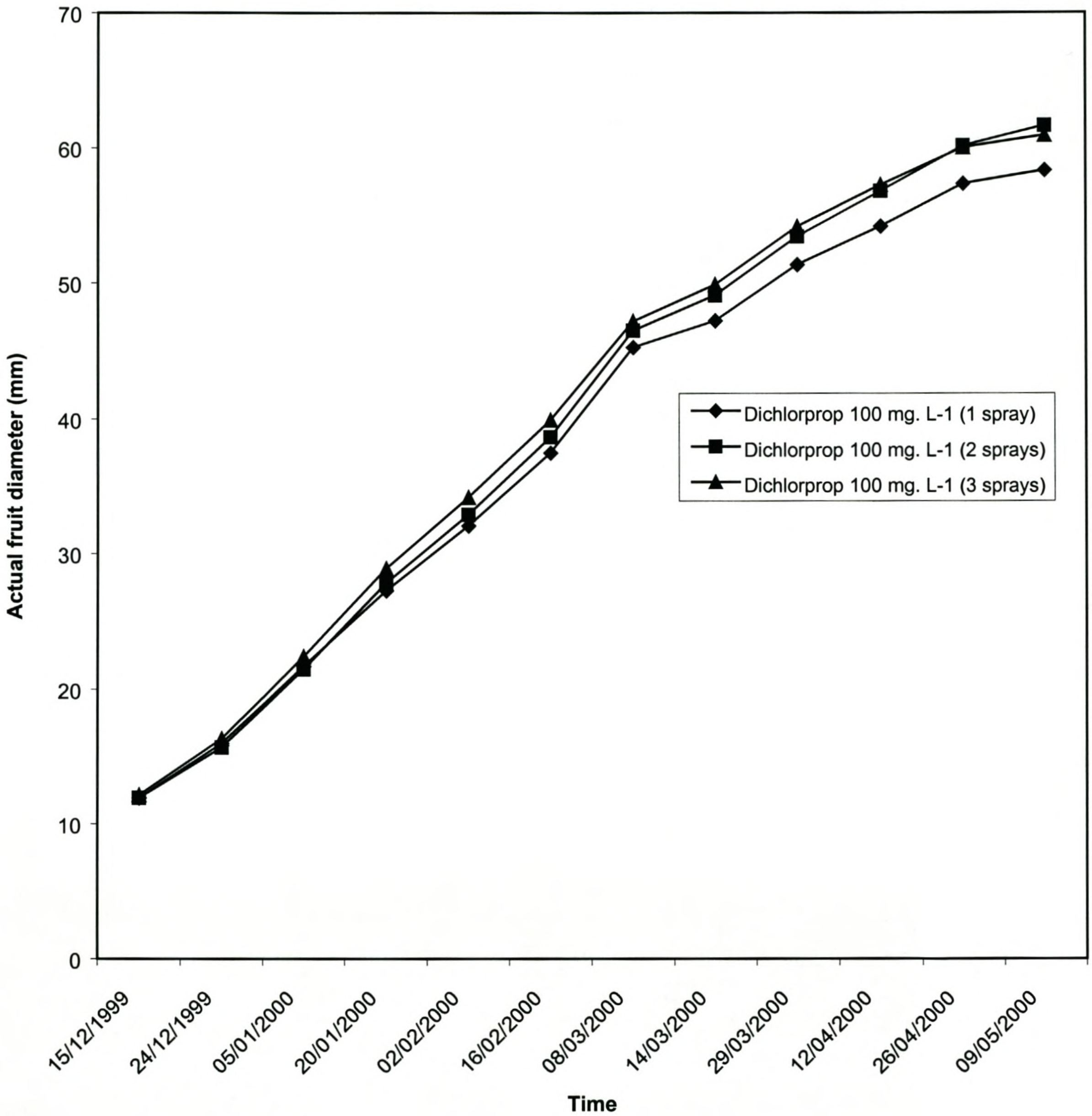

Figure 2. Fruit growth curves of 'Nules Clementine' mandarin sprayed with multiple dichlorprop sprays at Grabouw.

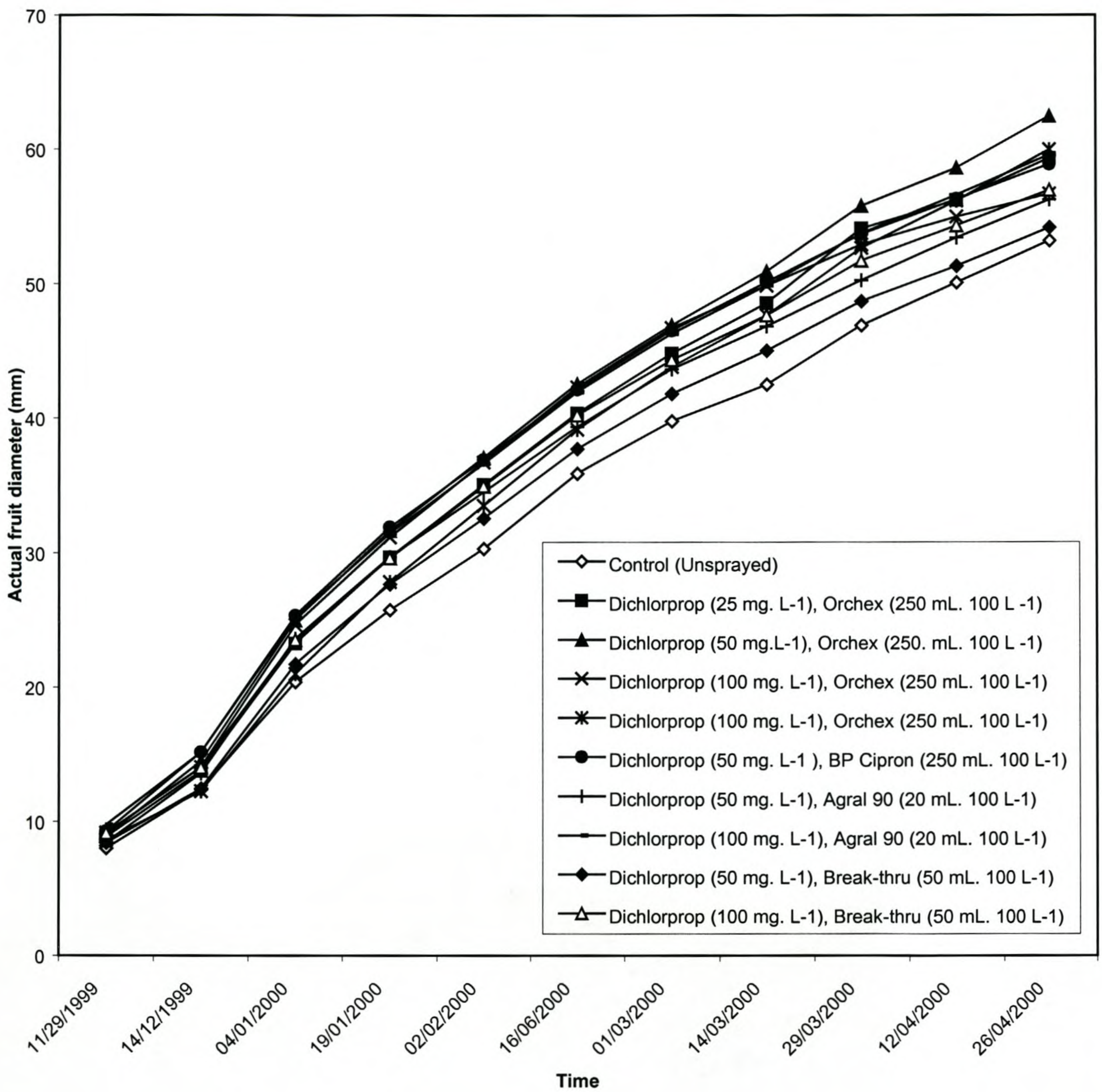

Figure 3. Fruit growth curves of 'Nules Clementine' mandarin sprayed dichlorprop in combination with different surfactants at Goedvertrouw, Stellenbosch.

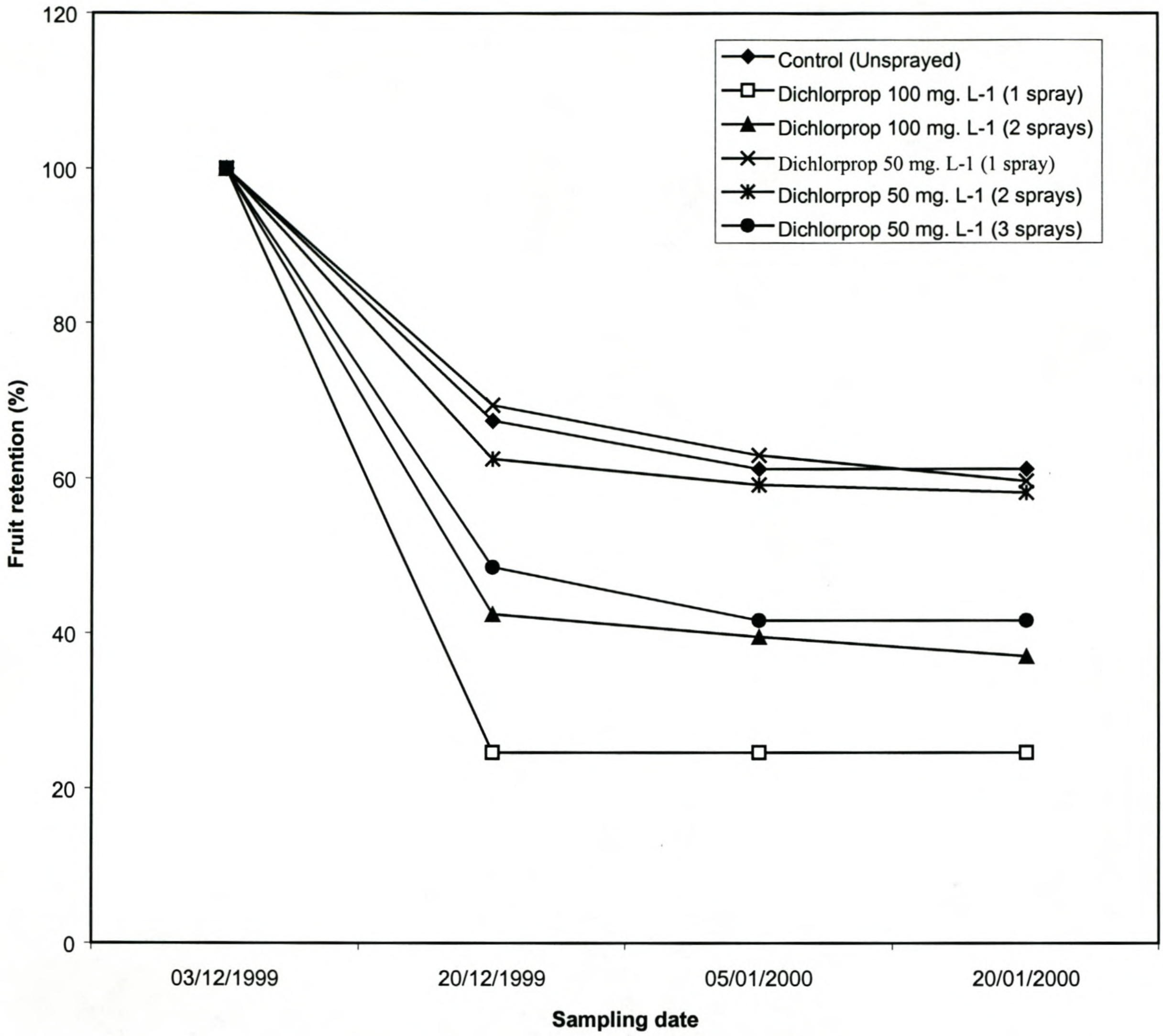

Figure 4. Fruit retention curves of 'Nules Clementine' mandarin after sprayed with multiple dichlorprop sprays at Welgevallen, Stellenbosch.

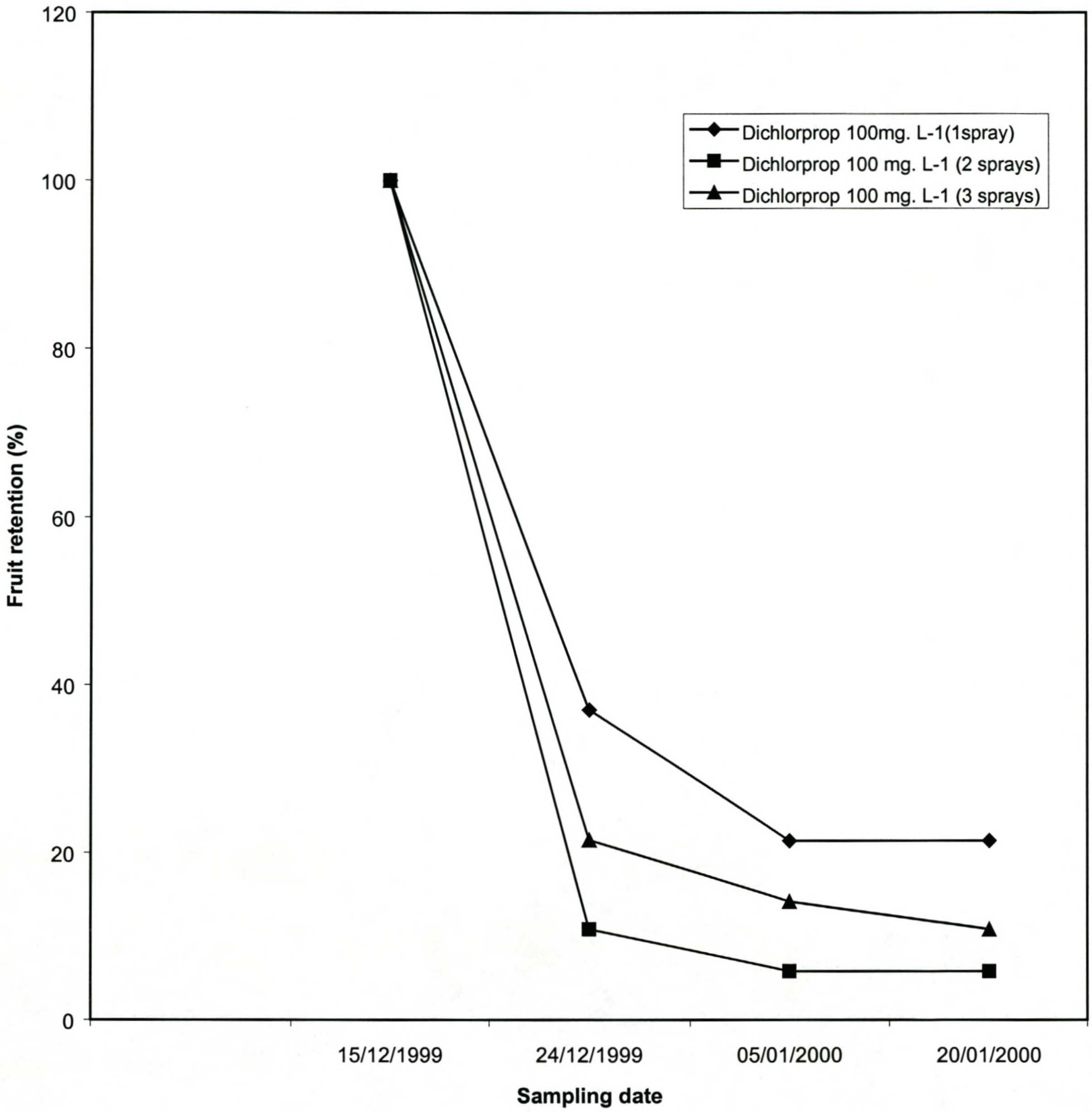

Figure 5. The retention curves of 'Nules Clementine' mandarin after sprayed with multiple dichlorprop sprays at Grabouw.

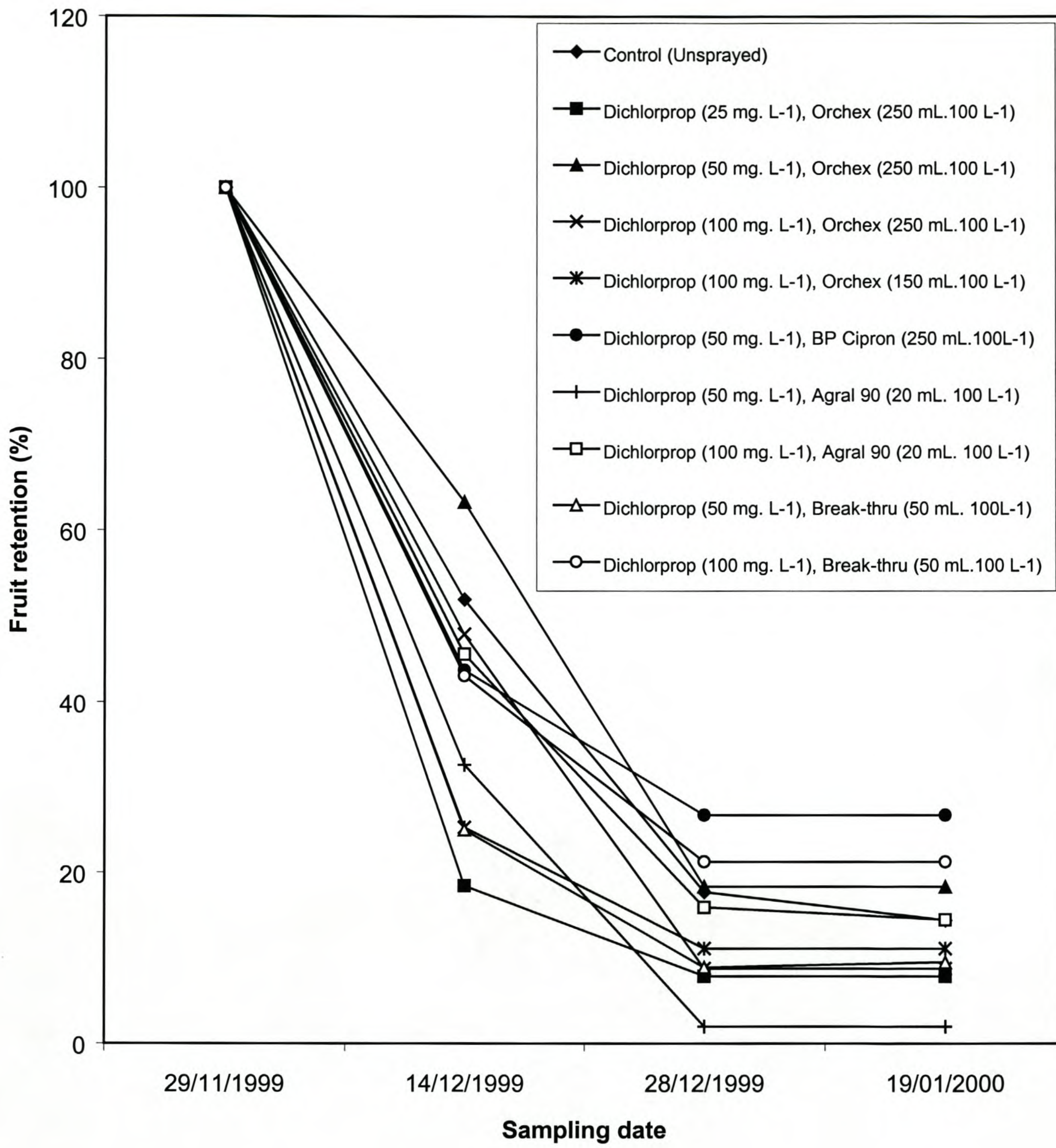

Figure 6. Fruit retention curve of 'Nules Clementine' mandarin after sprayed with dichlorprop in combination with different surfactants at Goedvertrouw, Stellenbosch.

6. PAPER 3: EFFECT OF DICHLORPROP IN COMBINATION WITH DIFFERENT SURFACTANTS ON FRUIT SIZE AND YIELD OF 'VALENCIA' ORANGE [*Citrus sinensis* (L.) Osbeck]

Abstract

Valencia orange [*Citrus sinensis* (L.) Osbeck] trees were sprayed with dichlorprop in combination with different surfactants at different concentrations, at the end of the physiological fruit drop period (early) and after the physiological drop period (late) to establish the best timing. During the first year (late application), there was no significant difference on fruit size, yield and internal fruit quality due to dichlorprop applications. However, during the second year (early and late applications) fruit size was significantly improved, especially by the early application of dichlorprop (50 and 100 mg. L^{-1}). The effects of dichlorprop in combination with different surfactants on internal fruit quality was inconsistent. However, there was a tendency of higher TSS in year one and significantly so in year two. Yield was drastically reduced up to 35% by early dichlorprop (25 mg. L^{-1}) with Orchex (200 mL. 100 L^{-1}) in the second year. Orchex and other mineral oils seemed to be more effective than Agral 90 at the same dichlorprop concentration and seems to have potential to reduce dichlorprop application costs.

Introduction

'Valencia' oranges have a tendency of bearing small fruit in South Africa (Blanke & Bower, 1992; Du Plessis & Koen, 1984). Therefore, its economic production has been limited since the export market requires fruit larger than 68 mm diameter. Gilfillan (1987) reviewed factors influencing fruit size in oranges and measures for fruit size improvement.

The Western Cape of South Africa has a cool climate and this limits the fruit size of 'Valencia' orange. The use of the synthetic auxin 2,4–dichlorophenoxy propionic acid (dichlorprop) to enhance fruit size has been reported to be successful in citrus (Agusti *et al.*, 1992) and more especially in small–fruited mandarin types (Miller, Bird & Maritz, 1999; Rabe, Koch & Theron, 1995). The dichlorprop effect on fruit size is the result of a direct fruitlet growth stimulation if sprayed just after the physiological fruit drop period (Agusti *et al.*, 1992), unlike other synthetic auxins such as NAA (Guardiola & Garcia–Luis, 2000) and 2,4-D (Stewart, Hield, Brannaman, 1952) where their effectiveness is mostly as a result of their thinning effect. With thinning, the fruit size enhancement by auxins is offset by a reduction of total tonnage. Dichlorprop can induce thinning when applied very early (before the end of the physiological fruit drop period) or when applied at high concentrations (Vanniere & Arcuset, 1992).

Dichlorprop is expensive and therefore the spray efficiency should be maximised as far as possible. Surfactants, chemical formulations that are used to enhance wetting by reducing the leaf surface tension, have been reported to improve spray effeciency (Swietlik & Faust, 1984). Rain after spray application often wash away sprayed chemicals thus reducing spray effeciency. Balneaves and Popay (1992) reported that spray droplets stick better to the leaves even after it rained if surfactants were used in spray application. Although the use surfactants showed good results in many trials, in South Africa the type of surfactants and concentration in combination with dichlorprop has not been established satisfactorily.

The aim of this study was to evaluate dichlorprop in combination with different surfactants at different concentrations on fruit size and yield. Early sprays of dichlorprop were applied during the physiological fruit drop period.

Materials and Methods

Plant materials and site selection. The study was conducted for two consecutive years at Citrusdal, region of the Western Cape, South Africa, (32°S, 19°E warm mesoclimate, altitude ca. 200 m) on fifty-year old 'Valencia' orange trees budded onto rough lemon (*C. jambhiri*) rootstock. The trees used for the study were selected for the uniformity of tree size and health.

Treatments and statistical design. After the physiological fruit drop period, dichlorprop was sprayed with handgun as a full cover spray on (24/11/1999) when the average fruit diameter was 19 mm. In the second year two spray timings were evaluated, i.e. early (10/11/2000) and late (22/11/2000) when the average fruit diameter was 8 mm and 12 mm, respectively. Treatments are outlined in Tables 1 and 3. The treatments were arranged in randomized complete block designs with ten replicates and ten single-tree replicates.

Fruit drop and fruit growth measurements. During the first year, one twig of the previous season's flush, 20 cm in length with approximately 20 fruit was tagged for fruit retention measurements. Fruitlets were counted at spray date and every two weeks thereafter until mid January. For fruit growth, 50 fruitlets per treatment were tagged at spray date (using replicates 1 to 5, 10 fruits per replicate, i.e. five on each side of the tree) and fruit growth was measured every two weeks until harvest.

Fruit size and yield. At harvest, fruit size and total yield were determined. Fruit size was determined by randomly collecting a sample of 15 to 20% of the yield and separating the fruit into size categories at a commercial packhouse on an electronic grader. Yield was determined as total fruit weight per tree.

Internal fruit quality. At harvest 12 fruits of the same size (six from each side of the tree) for each replicate were collected for internal fruit quality analysis. Fruit were squeezed using a hand reamer (citrus juicer). Juice was strained through two layers of Muslin cloth and juice content (%) was determined by subtracting the mass of the reamed peel from the total fruit mass. Total soluble solids (TSS) of the juice were determined by using an Atago hand refractometer; titratable acid (TA) was determined by titrating against 0.1 N NaOH using phenolphthalein as indicator and the TSS:TA was calculated.

Statistical analysis. The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyse the fruit size, yield and internal fruit quality data (SAS Institute Inc., 1990).

Results

Fruit drop and fruit growth. In general, the normal fruit drop pattern was slightly accelerated due to dichlorprop sprays (Figure 1). In the first year, with the spray being applied late, the effect on fruit growth observed due to dichlorprop spray applications was marginal (Figure 2).

[Figures 1 and 2]

Yield. Dichlorprop with different surfactants resulted in no significant effect on yield during the first year. However, during the second year, dichlorprop application resulted in a significant reduction in yield. Both early and late spray of dichlorprop (25 mg. L^{-1}) with Orchex (200 mL. 100 L^{-1}) resulted in a yield reduction of up to 50.2 kg (35%) when compared with the unsprayed control ($P< 0.16$).

[Tables 1 and 3]

Fruit size. During the first year, dichlorprop with different surfactants showed no effect on improvement of fruit size (Table 1). During the second year, early sprays of dichlorprop (50 and 100 mg. L^{-1}) with Orchex (200 mL. 100 L^{-1}) had a significantly higher proportion of large fruit (48 fruit per carton) compared to the unsprayed control (Table 3). All late dichlorprop treatments had a less dramatic effect on fruit size enhancement (Table 3). Orchex seemed to give better results than Agral 90 at the same dichlorprop concentration. Also, Orchex seemed to be effective at lower dichlorprop dosages, therefore showing a potential of reducing the dichlorprop application costs.

Internal fruit quality. Dichlorprop with different surfactants showed no consistent effect on juice percentage. During year one all dichlorprop treatments had marginally lower juice percentages (Table 2). During the second year, juice percentage was not affected by dichlorprop (Table 4). There was a tendency for higher TSS with dichlorprop in the first year (Table 2) and significantly so in year two (Table 4). Although there was variation in TSS and TA, it amounted to no effect on TSS:TA ratio in both years.

[Tables 2 and 4]

Discussion and Conclusion

There was inconsistent effect of dichlorprop applied with different surfactants on fruit size and yield in 'Valencia' orange. During the first year, a lack of fruit size improvement and no effect on yield was due to spray application being too late (sprayed on 24 November: 19 mm fruit diameter) to obtain the desired effect. During the second year, the late application (sprayed on 22 November: 12 mm fruit diameter) caused some fruit size enhancement while the early dichlorprop application (sprayed on 10 November: 8 mm fruit diameter) resulted in significantly larger fruit. Fruit size enhancement due to dichlorprop applications had been observed in other citrus cultivars (Agusti *et al.*, 1992; El-Otmani *et al.*, 1993; Rabe *et al.*, 1995; Vanniere & Arcuset, 1992). The best results were obtained when dichlorprop was sprayed at the end of cell division and the beginning of cell enlargement in 'Clementine' mandarin (Agusti *et al.*, 1992), and dichlorprop enhanced fruit size through cell enlargement rather than cell division in 'Fortune' mandarin (El-Otmani *et al.*, 1993). Therefore, dichlorprop application during the cell enlargement fruit growth stage (after physiological fruit drop period) amounts to less fruit size enhancement. Yield was not significanlty affected during year one but reduced significantly during the second year. Earlier dichlorprop applications are reported to induce fruit thinning, thus reducing total yield (Vanniere & Arcuset, 1992). Internal fruit quality was not significantly affected. Since the effect of dichlorprop in combination with surfactants on late application was not evident, the earlier applications seemed to favour Orchex than Agral 90 at the same dichlorprop concentration. When dichlorprop concentration doubled with Agral 90 it tended to give the same results as when with half the concentration in combination with Orchex. This implies that Orchex and other mineral oils as surfactant show a potential of reducing the dichlorprop application cost.

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Table 1. Effect of dichlorprop in combination with different surfactants on fruit size and yield of 'Valencia' orange at Citrusdal (2000). Trees were sprayed on 24/11/1999 (fruit diameter: 19 mm).

Treatments	Dichlorprop Conc. (mg. L $^{-1}$)	Surfactant Conc. (mL. 100 L $^{-1}$)	Fruit size count category per carton (%)				Total Yield (kg/tree)	Actual >88 count
			48-O/S	56-88	162-105	U/S		
Control (Unsprayed)			2.7 ab[z]	86.1 ab	11.4 bc	0.6 a	143.72 a	128.01 ab
Dichlorprop, Orchex	25	250	1.2 ab	80.8 bc	16.9 ab	0.2 ab	153.69 a	126.03 ab
Dichlorprop, Orchex	50	250	0.9 ab	88.1 a	9.8 c	0.4 ab	140.74 a	125.39 ab
Dichlorprop, Orchex	100	250	1.7 ab	80.6 bc	17.7 ab	0.4 ab	146.72 a	120.04 ab
Dichlorprop, Orchex	100	150	2.1 ab	82.8 ab	15.1 abc	0.1 ab	158.83 a	134.16 a
Dichlorprop, BP Cipron	50	250	3.4 a	75.4 c	21.3 a	0.2 ab	141.23 a	111.56 b
Dichorprop, Agral 90	50	20	1.0 ab	87.8 a	11.2 bc	0.1 ab	148.08 a	130.82 ab
Dichorprop, Agral 90	100	20	0.3 b	85.4 ab	14.3 bc	0.3 ab	153.29 a	131.50 ab
Dichlorprop, Break-thru	50	50	0.7 b	86.1 ab	13.2 bc	0.0 b	152.02 a	137.05 a
Dichlorprop, Break-thru	100	50	1.4 ab	86.9 ab	11.7 bc	0.2 ab	155.69 a	131.97 a
LSD (P=0.05)			2.7	6.6	6.6	0.6	21.4	20.1
Sign. Level			0.5159	0.0031	0.0188	0.6720	0.5746	0.2416

[z] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 2. Effect of dichlorprop in combination with different surfactants on internal fruit quality of 'Valencia' orange at Citrusdal (2000). Trees were sprayed on 24/11/1999 (fruit diameter: 19 mm).

Treatments	Dichlorprop Conc. (mg. L^{-1})	Surfactant Conc. (mL. 100 L^{-1})	Internal fruit quality			
			Juice %	TSS	TA	TSS:TA Ratio
Control (Unsprayed)			49.86 a[z]	11.20 bc	1.698 ab	6.62 ab
Dichlorprop, Orchex	25	250	47.92 ab	11.62 ab	1.706 ab	6.82 ab
Dichlorprop, Orchex	50	250	46.04 bc	11.28 abc	1.674 ab	6.75 ab
Dichlorprop, Orchex	100	250	46.58 bc	11.56 ab	1.748 ab	6.62 ab
Dichlorprop, Orchex	100	150	45.18 c	11.56 ab	1.688 b	6.88 a
Dichlorprop, BP Cipron	50	250	47.40 abc	11.42 abc	1.736 ab	6.59 a
Dichorprop, Agral 90	50	20	47.18 bc	10.94 c	1.620 ab	6.78 ab
Dichorprop, Agral 90	100	20	46.90 bc	11.42 abc	1.704 ab	6.73 a
Dichlorprop, Break-thru	50	50	47.98 ab	11.82 a	1.790 ab	6.63 ab
Dichlorprop, Break-thru	100	50	47.22 bc	11.58 ab	1.756 a	6.62 a
LSD (P = 0.05)			2.6	0.6	0.2	0.5
Sign. Level			0.0864	0.2331	0.6866	0.9549

[z] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 3. Effect of early and late sprays of dichlorprop in combination with different surfactants on fruit size and yield of 'Valencia' orange at Citrusdal (2001). Early spray was on 10/11/2000 (fruit diameter: 8 mm) and late spray was on 22/11/2000 (fruit diameter: 12 mm).

Treatments	Spray Dates	Dichlorprop Conc. (mg. L^{-1})	Surfactant Conc. (mL. 100 L^{-1})	Fruit size count category per carton (%)			Total yield (kg/tree)	Actual kg >88 count
				48–O/S	56-88	162-105		
Control (Unsprayed)		-	-	9.1 c[z]	77.9 a	13.0 a	143.2 a	124.6
Dichlorprop, Agral 90	Early	100	20	19.6 bc	70.4 abc	10.0 ab	111.1 ab	100.5
Dichlorprop, Orchex	Early	25	200	20.3 bc	70.6 abc	9.1 b	101.2 b	100.0
Dichlorprop, Orchex	Early	50	200	43.8 a	50.9 d	5.3 b	101.8 b	96.4
Dichlorprop, Orchex	Early	100	200	42.8 a	55.2 cd	2.0 b	110.4 ab	108.2
Control (Unsprayed)		-	-	16.8 bc	76.2 a	7.0 ab	109.5 b	101.8
Dichlorprop, Agral 90	Late	100	20	27.7 abc	64.8 abcd	7.5 ab	120.7 ab	111.6
Dichlorprop, Orchex	Late	25	200	31.6 ab	59.7 bcd	8.7 ab	93.0 b	84.9
Dichlorprop, Orchex	Late	50	200	13.0 bc	78.8 a	8.2 ab	117.5 ab	107.9
Dichlorprop, Orchex	Late	100	200	14.6 bc	73.1 ab	12.3 a	125.0 ab	109.6
LSD (P = 0.05)				18.8	16.5	8.8	32.9	
Sign. Level				0.0016	0.0061	0.3556	0.1578	
CONTRAST : (Pr > F)				**48-O/S**	**56-88**	**162-105**	**YIELD**	
Early vs Late				0.0272	0.0814	0.1334	0.3164	
Agral vs Orchex				0.3246	0.5521	0.3171	0.3813	
Dichlorprop Lin.				0.7066	0.8581	0.6366	0.0932	

[z] Numbers in columns with different letters are significantly different (P≤ 0.05, LSD).

Table 4. Effect of early and late dichlorprop sprays in combination with different surfactants on internal fruit quality of 'Valencia' orange at Citrusdal (2001). Early spray was on 10/11/2000 (fruit diameter: 8 mm) and late spray was on 22/11/2000 (fruit diameter: 12 mm).

Treatments	Spray Dates	Dichlorprop Conc. (mg. L^{-1})	Surfactant Conc. (mL. 100 L^{-1})	Internal fruit quality			
				Juice %	TSS	TA	TSS:TA Ratio
Control (Unsprayed)		-	-	40.64 a[z]	10.86 d	1.91 ab	5.75 a
Dichlorprop, Agral 90	Early	100	20	45.13 a	11.57 a	1.90 ab	6.14 a
Dichlorprop, Orchex	Early	25	200	43.24 a	11.42 abc	2.30 a	5.72 a
Dichlorprop, Orchex	Early	50	200	38.39 a	11.18 abcd	1.89 ab	6.07 a
Dichlorprop, Orchex	Early	100	200	41.91 a	11.10 dc	1.74 b	6.40 a
Control (Unsprayed)		-	-	44.81 a	11.16 bcd	1.87 ab	6.03 a
Dichlorprop, Agral 90	Late	100	20	41.00 a	11.27 abc	1.88 ab	6.07 a
Dichlorprop, Orchex	Late	25	200	40.08 a	11.22 abcd	1.91 ab	5.93 a
Dichlorprop, Orchex	Late	50	200	38.26 a	11.22 abcd	1.92 ab	5.90 a
Dichlorprop, Orchex	Late	100	200	39.72 a	11.54 ab	1.95 ab	5.99 a
LSD (P=0.05)				6.9	0.4	0.4	0.7
Sign. Level				0.4554	0.0219	0.5658	0.7377
CONTRAST: (Pr > F)				**JUICE%**	**TSS**	**TA**	**TSS:TA**
Early vs Late				0.1674	0.9564	0.6827	0.5249
Agral vs Orchex				0.1841	0.2342	0.6204	0.6039
Dichlorprop Lin.				0.9373	0.8508	0.1214	0.1290

[z] Numbers in columns with different letters are significantly different ($P \leq 0.05$, LSD).

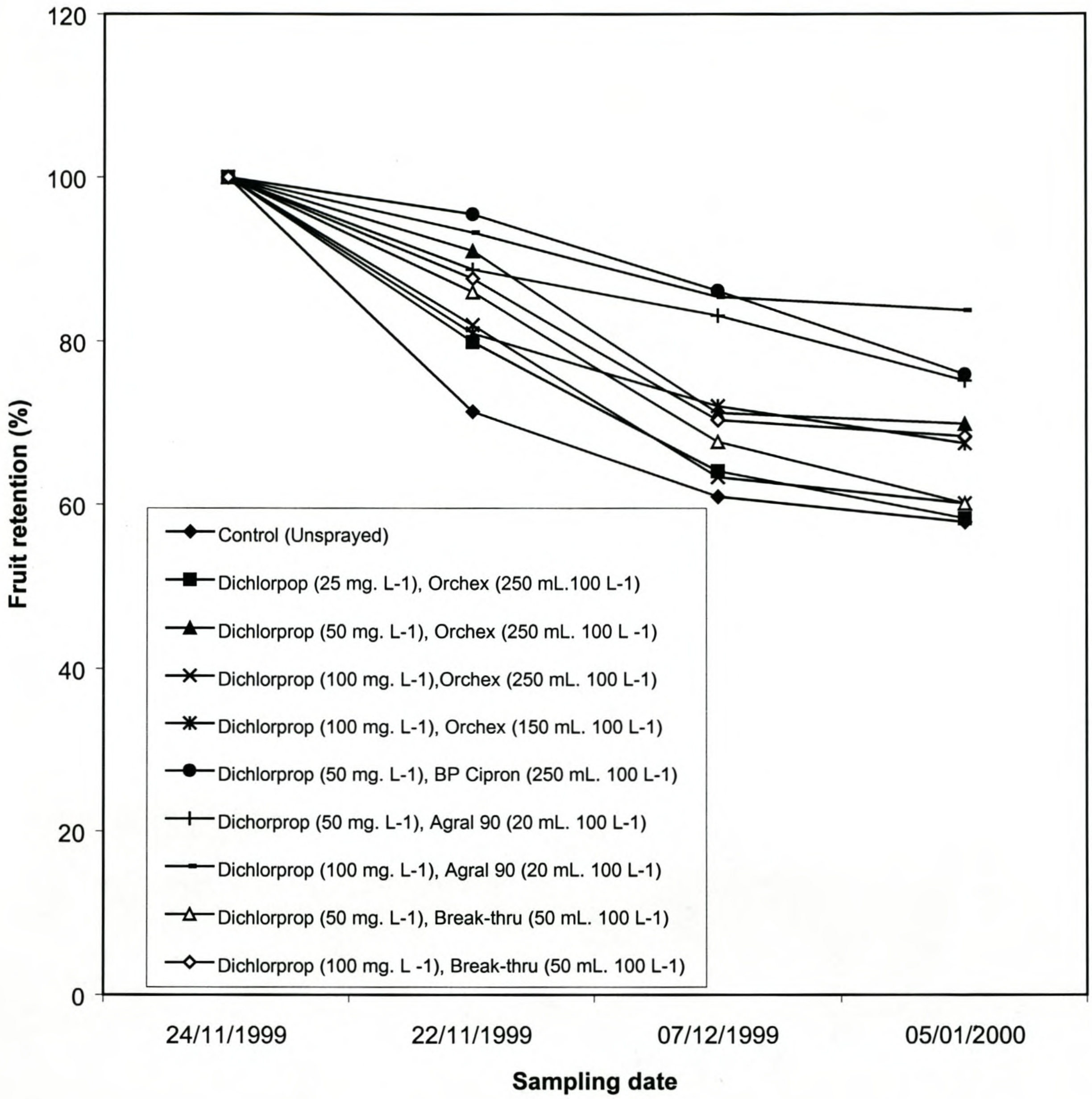

Figure 1. Fruit retention curves of 'Valencia' orange sprayed with dichlorprop in combination with different surfactants at Citrusdal.

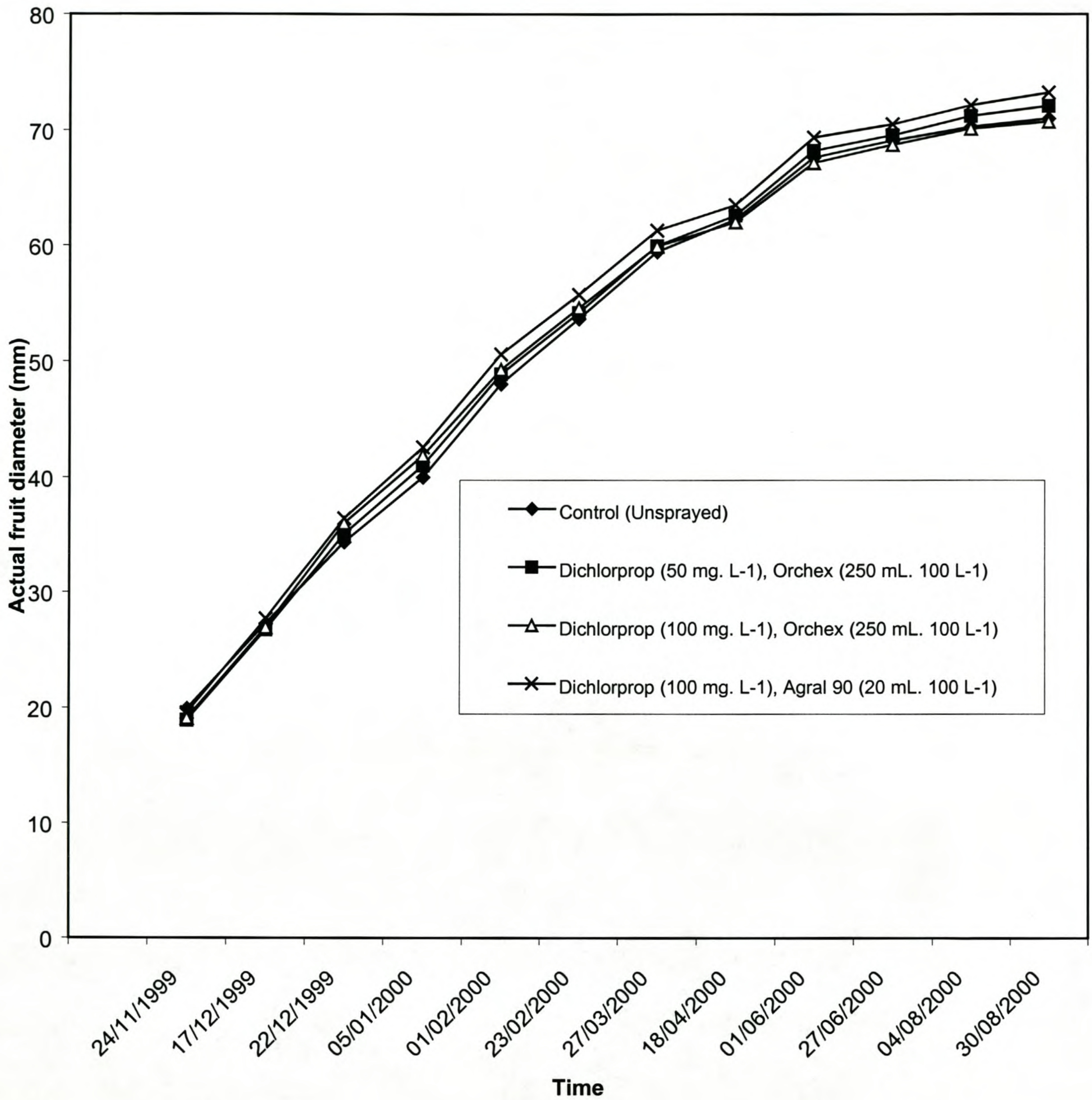

Figure 2. Fruit growth curves of 'Valencia' orange sprayed with dichlorprop in combination with different surfactants at Citrusdal.

7. PAPER 4: EFFECT OF DICHLORPROP APPLICATION AND LIGHT ON RIND CAROTENOID CONTENT IN CITRUS

Abstract

Total carotenoid content of the outer peel of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) and 'Valencia' orange [*C. sinensis* (L.) Osbeck] was evaluated during their fruit development to maturation stage. The evaluation was carried out in dichlorprop-sprayed and unsprayed fruit, both shaded and exposed fruit. In 'Nules Clementine' mandarin, dichlorprop–sprayed fruit showed no distinct trend in terms of carotenoid content. Also in the 'Valencia' orange trial, carotenoid content was inconsistent, but tended to be favoured by dichlorprop at 50 mg. L^{-1}. Fruit exposed to light showed a positive response on carotenoid accumulation compared with shaded fruit. Therefore, dichlorprop showed inconsistent effects on carotenoid accumulation in both 'Nules Clementine' mandarin and 'Valencia' orange. Our trials do not suggest that dichlorprop can be used to enhance peel carotenoid levels in citrus fruit. Futher reseach is required.

Introduction

With progressive fruit colour development, chlorophyll (green pigment) is gradually degraded and chloroplasts are transformed into carotenoid-rich chromoplasts (red, yellow or orange pigments) (Goldschmidt, 1988). Interconvension of chloroplasts to chromoplasts is governed by environmental (light and temperature), nutritional (nitrogen levels) and hormonal (gibberellins and cytokinins) signals (Spiegel-Roy & Goldschmidt, 1996). At cool temperatures root growth stops and levels of root growth substances decline, thus desired colour change be promoted (Young & Erickson, 1961). Good coloured fruit is an important aspect for the consumer since green colour is associated with immaturity.

As chlorophyll disappears carotenoid content of the rind becomes prominent. High light intensity promotes carotenoid synthesis in the rind; hence fruit growing in shaded areas of the tree tend to be paler than the exposed fruit (Coggins & Hall, 1975, Davies & Albrigo, 1994). The quantity of ß-citraurin is reduced at high temperatures (Stewart & Wheaton, 1971; Wheaton & Stewart, 1973). Therefore, when night temperatures are sufficiently low (down to 12.8°C) (Gilfillan, 1992), the chilling apparently causes mild injury to the rind and stimulates the production of stress related wound ethylene that promotes chlorophyll breakdown and unmasking of the underlying carotenoids (Cooper, Henry & Waldson, 1969). With this knowledge, ethylene-releasing compounds such as ethephon and ethychlozate had been widely used as pre-harvest sprays to degrade chlorophyll and concurrently expose the carotenoids (Iwahori, Tominanga & Oohata, 1986; Stewart & Wheaton, 1971). However, forced chlorophyll degradation is not often synchronized with carotenoid synthesis, but controlled by other factors, such as the accumulation of chlorophyllide a (Pons *et al.*, 1992)

The production of 'Nules Clementine' mandarin in the cool areas of the Western Cape region of South Africa (Mediterranean climate) experience warm mesoclimatic conditions towards the maturation period, thus restricting the unmasking of carotenoid pigments. The aim of this study was to quantify the total carotenoid content of the exocarp as influenced by 2,4 dichlorophenoxy propionic acid (dichlorprop, a synthetic auxin) applications, and light or shade during the late stages of fruit development in 'Nules Clementine' mandarin and 'Valencia' orange.

Materials and Method

Plant material and site selection. Two experiments were conducted in the Western Cape region of South Africa. (1) At Grabouw, (34°S, 19°E, winter rainfall area, altitude 305 m) on 'Nules Clementine' mandarin planted in 1993, budded onto 'Carrizo' citrange [*Poncirus trifoliata* (L.) Raf. x *C. sinensis*] rootstock and (2) at Citrusdal, (32° S, 20°E, warm mesoclimate, altitude 200 m), on fifty year-old 'Valencia' oranges budded onto rough lemon (*C. jambhiri*) rootstock. The trees used for study were selected for uniformity of tree size and health.

Treatments and statistical design. The trees selected comprised an experiment on enhancing fruit size using dichlorprop at different concentrations, viz. 0, 25, 50 and 100 mg. L^{-1} for 'Nules Clementine' mandarin and 0, 50, 100 mg. L^{-1} for 'Valencia' orange. For each tree used, 25 fruit were tagged and five fruit were picked at each sampling date. The fruit selection was based on uniformity of size and exposure to light. Fruit samples were analysed on the following dates: 28 February, 30 March, 18 April, 02 May and 15 May 2001 on 'Nules Clementine' mandarin and 30 August and 10 September 2001 on 'Valencia' orange. The experiments were arranged in a randomized complete block design with four and three treatments and five single-tree replicates, respectively. Another experiment was conducted on 'Nules Clementine' mandarin on trees forming part of a fruit sizing trial with dichlorprop at 0 and 100 mg. L^{-1}. Twenty fruit per tree were tagged, 10 fruit were shaded using paper bags and 10 fruits were exposed to light. At each sampling date, five fruit per treatment were harvested for carotenoid analysis. Sampled fruit were analyzed on the following dates: 28 February, 18 April and 15 May 2001.

Carotenoid analysis. In a room with lights off, fruit were carefully peeled, removing the flavedo (outer coloured layer) from the albedo (inner white layer) using a sharp knife, then macerated in a Waring Blender. Liquid nitrogen was used to facilitate maceration. On each sampling date, 1 g per five-fruit sample of finely ground rind in 10 ml of 100% acetone was kept for 1 hour in a refrigerator. Thereafter the flavedo-acetone mixture was stirred using a Vortex-2 Genie electric stirrer and centrifuged at 10 x 1000 rpm for 15 minutes. The extract was then analyzed by spectrophotometer at 670, 645 and 470 λ. Total carotenoid content was calculated from the extract values according to Lichtenthaler's equation (Lichtenthaler, 1987).

Statistical analysis. The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyze the carotenoid content data (SAS Institute Inc., 1990).

Results and Discussion

Dichlorprop. Fruit from dichlorprop-treated 'Nules Clementine' mandarin trees showed no consistent trends in total carotenoid content (Figure 1). However, dichlorprop concentration at 50 mg. L^{-1} was observed to be higher, but not statistically than other treatments throughout the experiment. The total carotenoid content of the rind from dichlorprop-treated 'Valencia' orange trees, tended to be favoured by 50 mg. L^{-1}, but no significant difference could be established at harvest (Figure 2). In previous research, the synthetic auxin 2,4-D had little or no effect on enhancing carotenoid content of the rind but delayed chlorophyll degradation (Coggins & Jones, 1977). Data obtained by Koch (1995) indicated that dichlorprop at 100 mg. L^{-1} enhanced fruit colouring of 'Clementine' mandarin based on the selective picking percentage. However, in his study the carotenoid content was not quantified. Even though auxins of all types stimulate many kinds of plant cells to produce ethylene (Salisbury & Ross, 1992), ethylene induces a chlorophyll degrading enzyme, chlorophyllase and has no effect on carotenoid synthesis (Trebitsh, Goldschmidt & Roiv, 1993). Futhermore, Pons *et al*. (1992) indicated that forced chlorophyll degradation using ethephon was not synchronized with carotenoid synthesis. Therefore, results obtained in this study balanced the previous report by Koch (1995) that dichlorprop has a potential of enhancing fruit colouration through carotenoid synthesis.

[Figures 1 and 2]

Shading. Exposed fruit tended to have higher total rind carotenoid content than shaded fruit (Figure 3). Total carotenoid content was observed to be significantly higher in exposed fruit compared to shaded fruit, both sprayed with dichlorprop at 100 mg. L^{-1}. However, there was no significant difference between the unsprayed controls both exposed and shaded fruit, but, exposed fruit tended to obtain higher carotenoid levels.

[Figure 3]

In conclusion, dichlorprop showed no consitent trends on total rind carotenoid content. Shaded fruit had lower total carotenoid content of the rind than exposed fruit.

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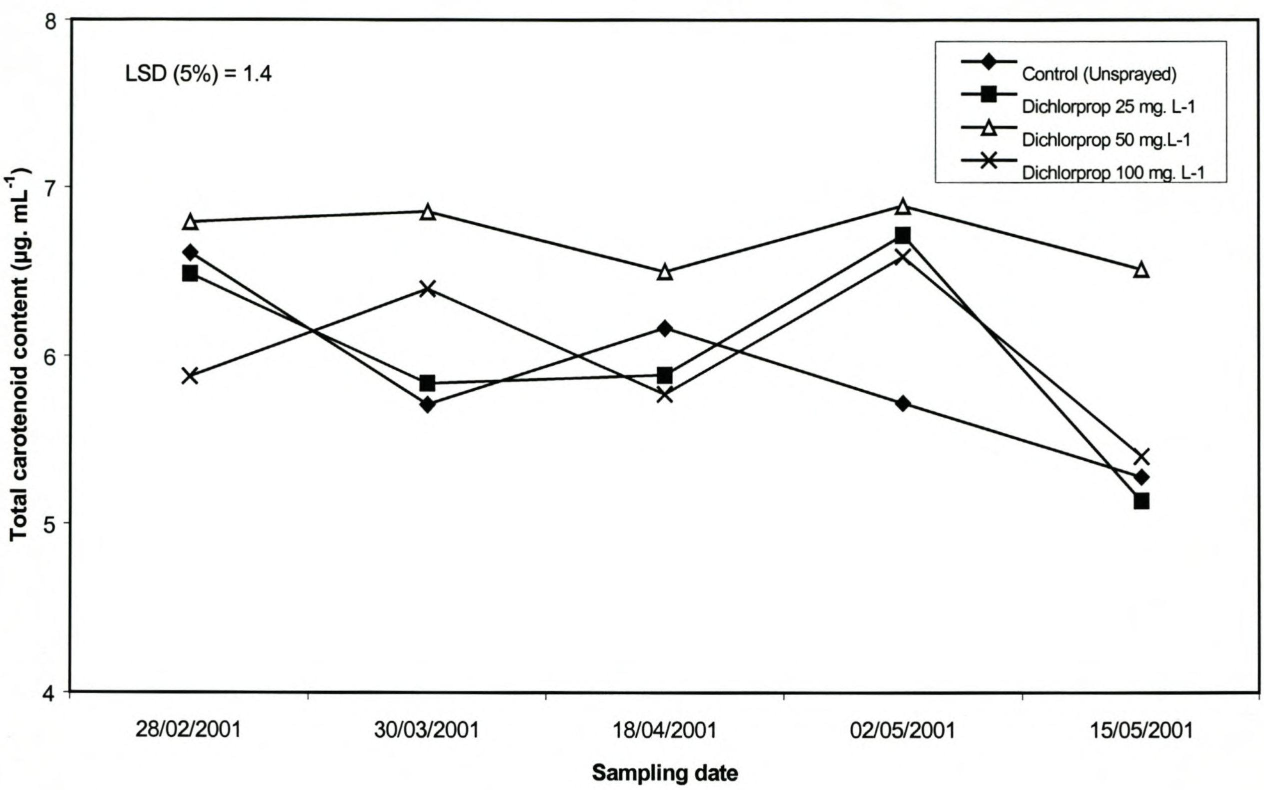

Figure 1. Total carotenoid content of 'Nules Clementine' mandarin rind, sampled over the later part of the growing season as influenced by dichlorprop spray applications at Grabouw.

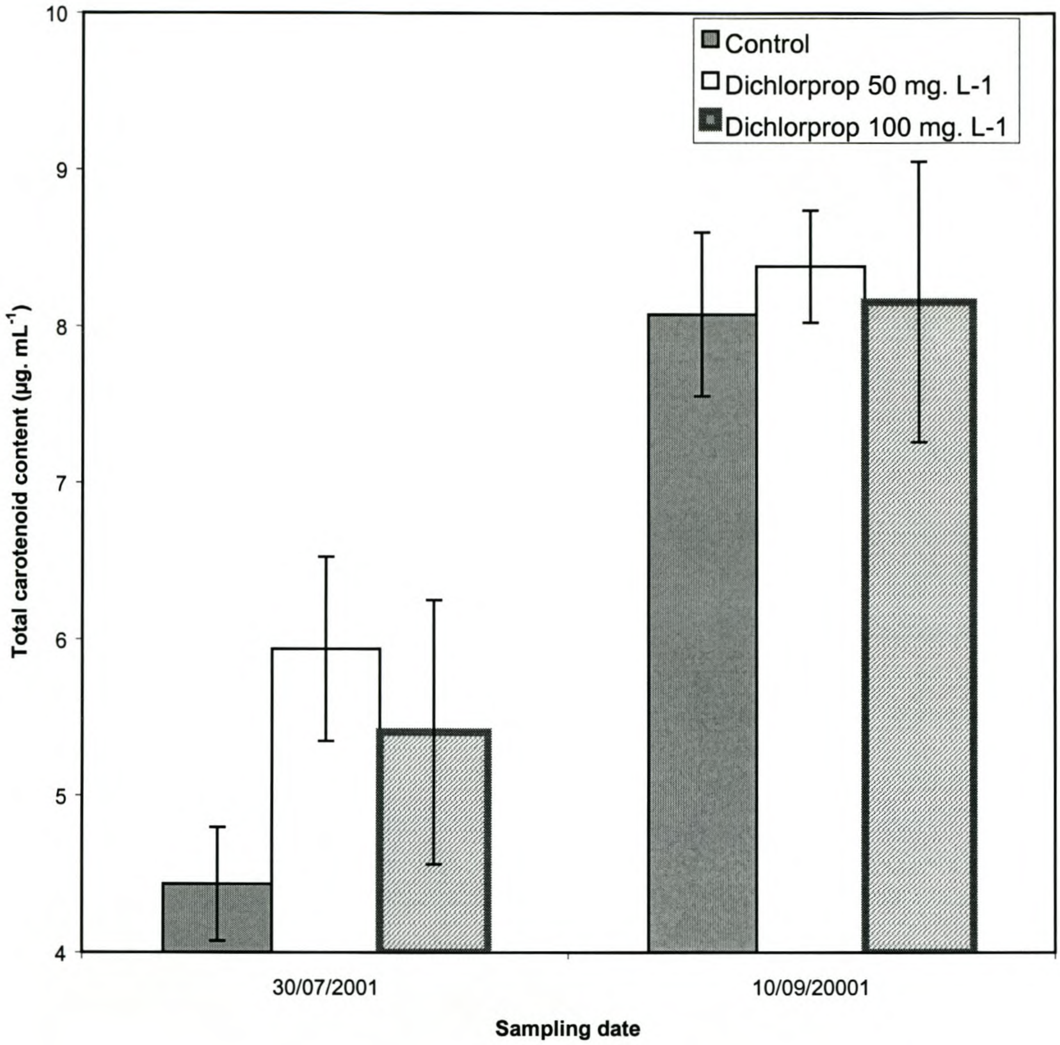

Figure 2. Total carotenoid content of 'Valencia' orange rind, sampled over the later part of the growing season as influenced by dichlorprop spray applications at Citrusdal.

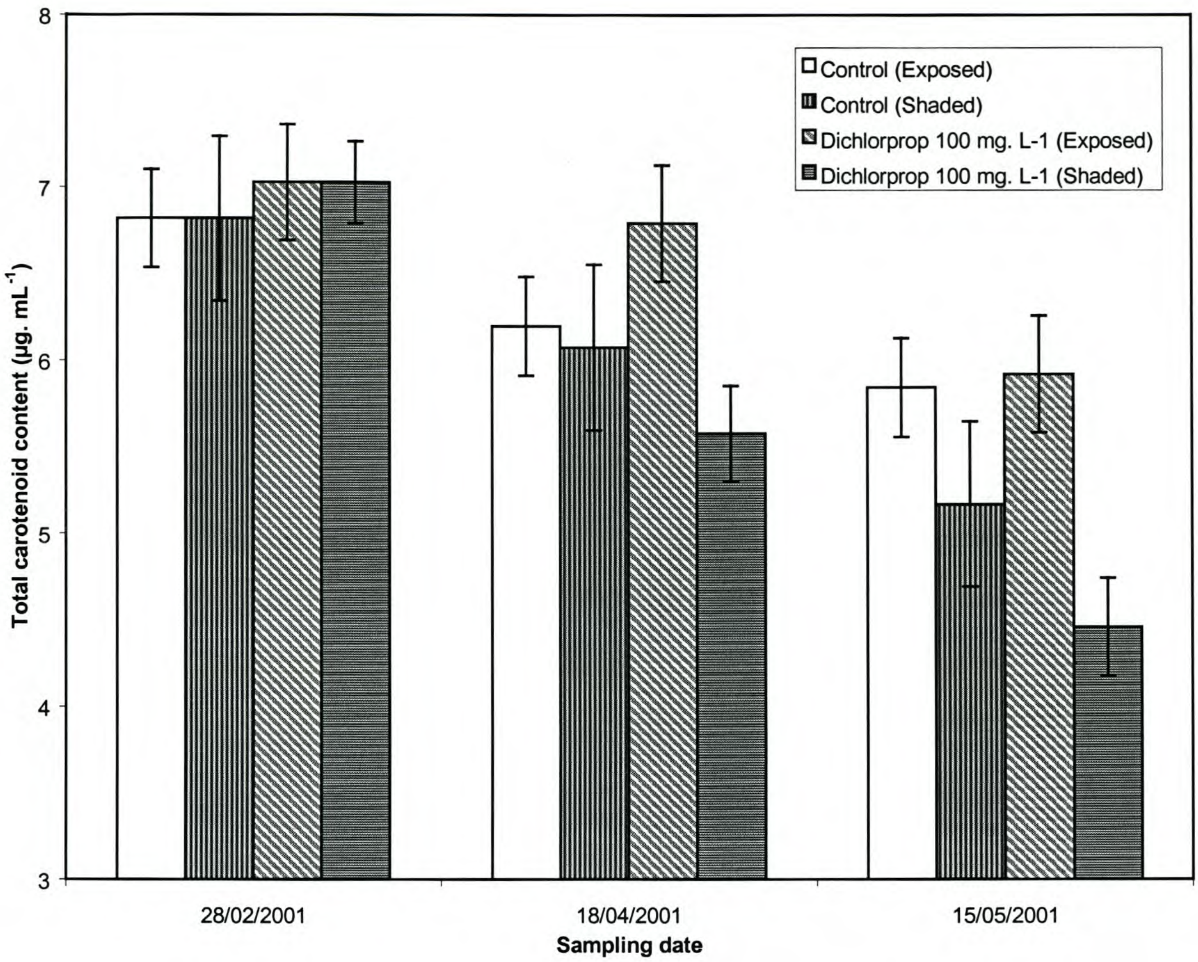

Figure 3. Total carotenoid content of 'Nules Clementine' mandarin rind, as influenced by light and dichlorprop spray applications at Grabouw.