STUDIES TO REDUCE THE SIZE OF THE NAVEL-END OPENING OF NAVEL ORANGES

BY

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DECLARATION

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SUMMARY

The size of the navel-end opening is an important parameter for external fruit quality in navel oranges [*Citrus sinensis* (L.) Osbeck]. The application of 2,4-dichlorophenoxyacetic acid (2,4-D) to increase the percentage of closed navel-ends and reduce the size of the navel-end opening was conducted on six different navel orange cultivars. Treatments were applied at full bloom (FB), 100% petal drop (PD), as well as 2 weeks (2 WAPD) and 4 weeks after 100% petal drop (4 WAPD), at 15 $mg \cdot L^{-1}$ to 45 $mg \cdot L^{-1}$, to determine the most effective timing and concentration. The application of 2,4-D at FB consistently decreased the average navel-end size (all fruit) and increased the percentage of closed navel-ends in all the cultivars, with later applications at PD, 2 WAPD and 4 WAPD being generally ineffective, regardless of the concentration applied. There were no major negative side effects on internal and external fruit quality, except for the reduction in juice content (%), especially with the later treatments. Therefore, 15 $mg \cdot L^{-1}$ 2,4-D at FB can be applied to increase the percentage of closed navel-ends and possibly increase export packouts.

Navel oranges have a small secondary fruit located inside the primary fruit at the stylar-end and an opening at the stylar-end called the navel-end opening or the stylar-end aperture. Fruit growth and development was studied in three navel orange cultivars by measuring the primary fruit diameter, the secondary fruit diameter and the navel-end opening fortnightly, using both destructive and non-destructive sampling methods. The relationships between the primary fruit size, the secondary fruit size and the navel-end opening size were studied using correlation analysis. In addition, the effect of 2,4-D on fruit morphology, when applied as a treatment to reduce the size of the navel-end opening, was also evaluated on the same cultivars. The primary fruit, the secondary fruit and the navel-end opening followed a similar developmental pattern, although the navel-end opening developed later, about six weeks after FB. The primary fruit size was not related to the size of the secondary fruit or the navel-end opening. Similarly, the size of the navel-end opening was not related to the size of the secondary fruit. No negative effects were noted on the primary fruit morphology when 2,4-D was applied.

Fruit splitting is a major physiological disorder of ‘Marisol’ Clementine mandarin (*Citrus reticulata*) fruit. The effect of application of 2,4-D on fruit splitting and fruit quality was evaluated on ‘Marisol’ Clementine mandarin trees grafted on Troyer citrange rootstock. Treatments included an untreated control, 2,4-D applied at 15 $mg \cdot L^{-1}$ or 25 $mg \cdot L^{-1}$ at FB and 15

mg·L^{-1} or 25 mg·L^{-1} at PD. The application of 2,4-D reduced fruit splitting in 'Marisol' Clementine fruit. Internal fruit quality was not affected by the treatments, however, the fruit developed a coarse rind due to enlarged oil glands and the styles stayed attached on the fruit until harvest. Therefore, although 2,4-D reduced fruit splitting, it cannot be recommended at the timings and concentrations evaluated.

OPSOMMING

STUDIES OM DIE NAWEL-ENT GROOTTE VAN NAWEL LEMOENE TE VERKLEIN

Die grootte van die nawel-ent opening is ‘n belangrike parameter vir eksterne vrugkwaliteit van nawel lemoene [*Citrus sinensis* (L.) Osbeck]. Die toediening van 2,4-dichlorofenoksie asynsuur (2,4-D) om die persentasie geslote nawel-ente te vermeerder en die grootte van die nawel-ent opening te verklein is uitgevoer op ses verskillende nawel lemoen kultivars. Behandelings is toegedien by volblom (FB), 100% blomblaarval (PD), asook 2 weke (2WAPD) en 4 weke na 100% blomblaarval (4 WAPD), teen 15 $mg \cdot L^{-1}$ tot 45 $mg \cdot L^{-1}$, om die mees effektiewe tyd van toediening en konsentrasie te bepaal. Die toediening van 2,4-D by FB het herhaaldelik die gemiddelde nawel-ent grootte (alle vrugte) verminder en die persentasie geslote nawel-ente vermeerder in al die kultivars, terwyl die later toediening by PD, 2 WAPD en 4 WAPD oor die algemeen nie effektief was nie, ongeag die konsentrasie toegedien. Daar was geen noemenswaardige negatiewe effekte op interne en eksterne vrugkwaliteit nie, behalwe vir ‘n verlaging in die sapinhoud (%) van vrugte, veral by die later behandelings. Dus kan 15 $mg \cdot L^{-1}$ 2,4-D by FB toegedien word om die persentasie geslote nawel-ente te vermeerder en moontlik ook die uitvoerpersentasie te verhoog.

Nawel lemoene het ‘n klein sekondêre vrug binne die primêre vrug aan die styl-ent en ‘n opening by die styl-ent wat die nawel-ent opening of die styl-ent opening genoem word. Die vruggroei en ontwikkeling van drie nawel kultivars is bestudeer deur die primêre en sekondêre vrugdeursnit en die nawel-ent opening elke twee weke te meet, deur gebruik te maak van destruktiewe en nie-destruktiewe monsterneming. Die effek van 2,4-D op vrugmorfologie, toegedien as ‘n behandeling om die nawel-ent grootte te verklein, is ook ge-evalueer op dieselfde kultivars. Die primêre vrug, die sekondêre vrug en die nawel-ent opening het dieselfde ontwikkelingspatroon gevolg, alhoewel die nawel-ent opening later ontwikkel het. Daar was geen sterk verwantskap tussen die primêre vruggrootte en die sekondêre vruggrootte of die grootte van die nawel-ent opening nie. Daar was ook nie ‘n vewantskap tussen die grootte van die nawel-ent opening en die sekondêre vruggrootte nie. Geen negatiewe effekte op vrugmorfologie as gevolg van die 2,4-D toediening is waargeneem nie.

Vrugsplit is ‘n belangrike fisiologiese abnormaliteit van ‘Marisol’ Clementine (*Citrus reticulata*) vrugte. Die effek van 2,4-D op vrugsplit en vrugkwaliteit is ge-evalueer op ‘Marisol’ Clementine mandaryn bome op Troyer citrange onderstamme. Die behandelings het ‘n onbehandelde kontrole, 2,4-D toegedien teen 15 $mg \cdot L^{-1}$ of 25 $mg \cdot L^{-1}$ by FB en 15 $mg \cdot L^{-1}$ of 25 $mg \cdot L^{-1}$ by PD ingesluit. Die toediening van 2,4-D het vrugsplit verminder. Interne vrugkwaliteit was nie geaffekteeer deur die behandelings nie, maar die vrugte het ‘n growwe skil ontwikkel as gevolg van vergrote oliekliere en die style het aangeheg gebly aan die vrugte tot oestyd. Dus, alhoewel 2,4-D vrugsplit verminder het, kan dit nie aanbeveel word teen die tyd van toediening en konsentrasie soos ge-evalueer in hierdie studie nie.

Dedicated to the memory of my late parents Gavin and Vaidah, and my late brother Sebastian

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This thesis was written according to the language and style required by the journals of the *American Society for Horticultural Science*. Each chapter represents an individual paper and some repetition between the chapters may occur.

1. LITERATURE REVIEW

STUDIES TO REDUCE THE SIZE OF THE NAVEL-END OPENING OF NAVEL ORANGES

1. INTRODUCTION

Navel oranges [*Citrus sinensis* (L.) Osbeck] have a small secondary fruit located within the primary fruit at the stylar-end (Davies, 1986). The secondary fruit can develop within the primary fruit or in some cases may protrude outside the primary fruit, resulting in the navel-end opening, also called the stylar-end aperture (Davies, 1986). The size of the navel-end opening is one of the parameters that are used for external fruit quality criteria during packing and export of navel oranges (Verreynne, 2008). The maximum acceptable navel-end diameter for export fruit is 20 mm (Grout, 1992). Fruit with large open navel-ends can only be sold on the local market where it earns low prices which in some cases do not cover production costs of the grower.

Certain problems in the production of navel oranges may be associated with the presence of the secondary fruit and the size of the navel-end opening. The incidence of stylar-end decay seems to be related to the size of the navel-end opening (Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939, 1941). Physiological disorders such as fruit splitting have also been linked to the size of the navel-end opening in citrus (Krezdorn, 1969; Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939). Large navel-end openings also provide an entry point and harboring place for insects making their control difficult (Soule and Grierson, 1986). Secondary fruit yellowing (SFY) which is caused by the abscission of the secondary fruit from the primary fruit is a problem in the production of navel oranges (Lima and Davies, 1984a). Fruit affected by SFY usually abscises from the tree before maturity (Lima and Davies, 1984a).

Various factors have been associated with the formation of a large open navel-end. Climate has an effect on the size of the navel-end opening (Soule and Grierson, 1986; Wager, 1941). Wager (1939) reported that weather after fruit set may influence the size of the navel-end. The bearing position of fruit on the tree also affects the size of the navel-end opening (Lima and Davies, 1984a; Wager, 1939). Cameron and Frost (1968) reported that in ‘Washington’ navel orange there is a greater tendency for smaller navel-end openings in fruit from nucellular

seedlings than those from seed parent trees. In addition, differences in navel-end size have been noted between navel orange cultivars, for instance 'Bahianinha' navel orange has a smaller navel-end opening compared to 'Washington' navel orange (Saunt, 2000).

The reduction of the size of the navel-end opening would bring several advantages in the production of navel oranges. Smaller navel-ends will produce higher export packout, reduce fruit splitting and stylar-end decay. In addition, insect control would also be more effective in fruit that have smaller or closed navel-ends. Presently, there is no commercial solution in citrus production for the reduction of the navel-end size. Krezdorn (1969) reported that dipping flowers in a solution containing 20 $mg \cdot L^{-1}$ 2,4-dichlorophenoxyacteic acid (2,4-D) and 250 $mg \cdot L^{-1}$ gibberellic acid (GA_3) reduced the size of the navel-end. Recent work by Gardiazabal (2006) and Saveedra (2006) has shown that application of 2,4-D at full bloom significantly increased the percentage of fruit with closed navel-ends and significantly reduces average navel-end size. Preliminary studies in South Africa have shown that the application of 25 $mg \cdot L^{-1}$ 2,4-D at 100% petal drop increased the percentage of closed navel-ends and reduced the average navel-end size in 'Palmer', 'Robyn' and 'Lane Late' navel orange (Verreynne, 2008). The opportunities presented by the previous work for navel-end size reduction or complete navel-end elimination will be exploited in this study.

2. THE NAVEL ORANGE

Navel oranges are believed to have developed from a mutation of the sweet orange (Davies, 1986). The feature that distinguishes them from other sweet oranges is the presence of a secondary fruit located at the stylar end of the primary fruit (Fig. 1). This characteristic is also sometimes found in mandarin hybrids, some grapefruit and other citrus although its presence is never consistent (Davies, 1986; Saunt, 2000; Soule and Grierson, 1986). For example, in certain types of mandarins the secondary fruit appears as a small embryonic fruit and is usually enclosed by the rind of the primary fruit (Spiegel-Roy and Goldschmidt, 1996). The secondary fruit is nearly always present in navel oranges, varying in size according to the cultivar and conditions under which the fruit develops (Davies, 1986). For example in 'Washington' navel orange the secondary fruit may develop up to a diameter of 20 to 30 mm and in some cases might protrude slightly from the primary fruit (Spiegel-Roy and Goldschmidt, 1996). In the flowers of navel oranges, tertiary structures have also been found but these do not develop into tertiary fruit (Soule and Grierson, 1986).

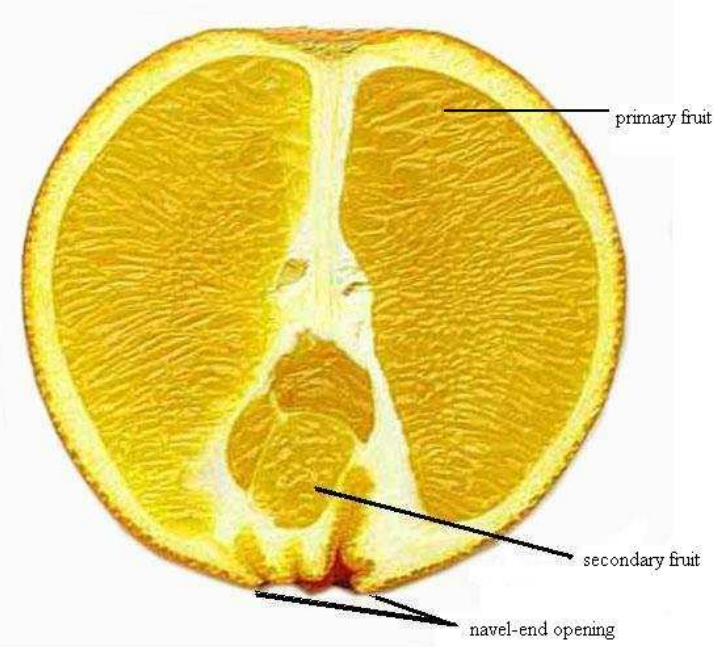

Fig. 1. Longitudinal section of the navel orange showing the primary fruit, the secondary fruit and the navel-end opening

The production of navel oranges presents some advantages to the grower over other sweet orange cultivars like Valencias. They are generally an early maturing group forming an important source of income at the start of the harvest season for citrus growers worldwide (Lima et al., 1980). In addition, they are seedless due to the production of non-viable pollen which makes them excellent dessert fruit (Davies, 1986). They are grown primarily for the fresh market because of their superior eating quality and are highly priced as dessert fruit (Davies, 1986; Saunt, 2000). However, if processed, the compound limonin is released which results in bitterness of the juice which makes navel oranges less suitable for processing (Saunt, 2000).

Navel oranges are more specific in their climatic adaptability compared to other sweet oranges and are generally more vulnerable to environmental stresses (Davies, 1986; Saunt, 2000). This puts limitations on their production especially in the case where high quality fruit are required. Mediterranean type climates with warm days and cool nights produce fruit of high eating quality (Davies, 1986; Saunt, 2000). In South Africa, the Western Cape with its Mediterranean type climate is most suitable for production of high quality navel oranges and is the largest production area of navel oranges (35,5%) with the Eastern Cape (27.8%) the second largest (CGA, 2007).

2.1 Primary fruit: morphology and development

Morphologically the primary fruit in navel oranges is similar to other sweet orange fruit. It is a special type of berry called a hesperidium and is considered a true fruit as it arises through growth and development of the ovary. Citrus fruits are made up of two distinct tissues, the endocarp, which is the edible part and the pericarp also known as the rind (Spiegel-Roy and Goldschmidt, 1996). The endocarp is made up of segments filled with juice vesicles (Spiegel-Roy and Goldschmidt, 1996).

Development of the primary fruit in the navel orange is very similar to that of other sweet oranges. Bouma (1959) and Holtzhausen (1969) studied the development of the 'Washington' navel orange fruit and reported that it followed a sigmoidal pattern with three growth stages (Fig. 2). In stage 1, fruit growth is slow and occurs due to cell division. Stage 2 is characterized by rapid fruit growth with cell enlargement and cell differentiation predominating. Stage 3 is regarded as the maturation period characterized by slowing down of the fruit growth rate. The growth stages are the same as for Valencia sweet orange (Bain, 1958), showing that there is no difference in the growth pattern of sweet oranges with the presence or absence of the secondary fruit.

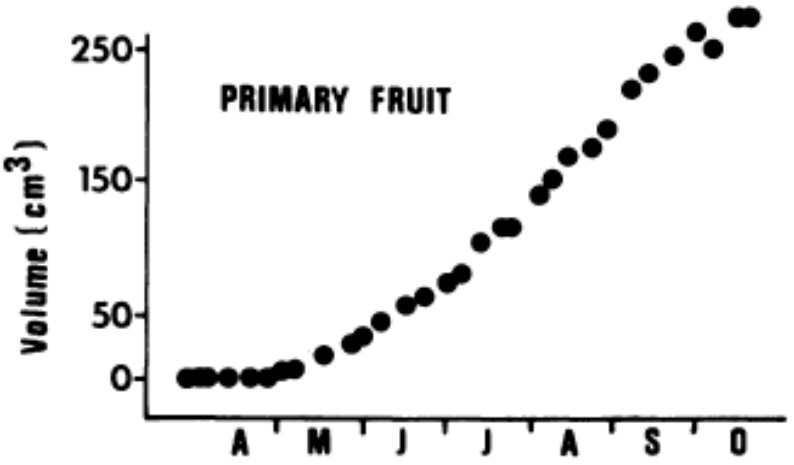

Fig. 2. Change in the primary fruit volume of the navel orange from anthesis until fruit maturity in the northern hemisphere (Lima and Davies, 1984b).

2.2 Secondary fruit: morphology and development

Morphologically the secondary fruit is very diverse. It can range in size from hardly noticeable rind tissue to a well developed fruit that is similar to the primary fruit but only

smaller in size (Lima and Davies, 1984b). In some cases, juice sacs and a central axis are found in fully developed secondary fruit (Lima and Davies, 1984b). Coit and Hodgson (1919), (cited in Lima and Davies, 1984b) stated that the central axis of secondary fruit is an extension of the primary fruit axis and functions as a pedicel for the secondary fruit. The secondary fruit is located within the primary fruit at the stylar-end and may sometimes protrude outside the primary fruit depending on cultivar and growing conditions (Davies, 1986; Lima and Davies, 1984b).

Extensive studies on the growth and development of the secondary fruit were conducted by Lima and Davies (1984b). Development of the secondary fruit can be traced back to the period of floral initiation in citrus. Prior to anthesis the secondary gynoecium begins to develop within the primary one (Davies, 1986; Lima and Davies, 1984b). When the flower buds are about 1.5 to 2 mm long, the secondary fruit begins to develop as a whorl of secondary carpel primordia within the primary ovary (Lima and Davies, 1984b). Secondary carpels are easily distinguishable when the flower buds are 6 to 8 mm long (Davies, 1986). The development of flower parts in secondary fruit is not perfect as the stigma and style of the secondary fruit are not as distinct as those of the primary fruit (Lima and Davies, 1984b). The cell enlargement stage in secondary fruit starts two weeks later than that of the primary fruit (Fig. 3) but the secondary fruit also follows the same sigmoidal developmental pattern as the primary fruit (Lima and Davies, 1984b).

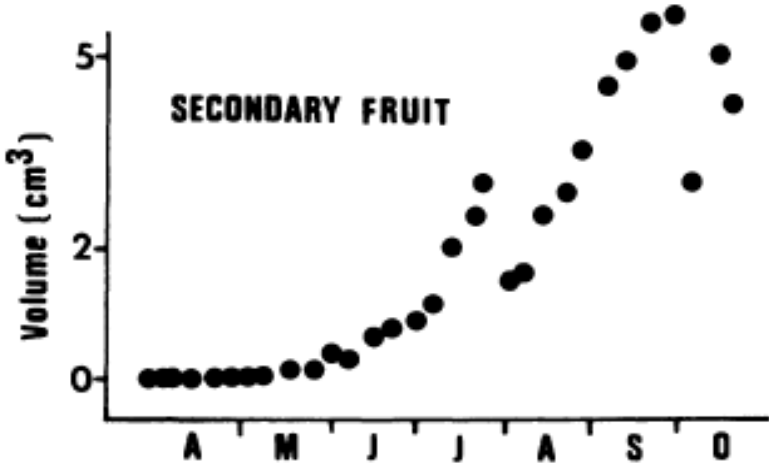

Fig. 3. Change in the secondary fruit volume of the navel orange from anthesis until fruit maturity in the northern hemisphere (Lima and Davies, 1984b).

2.3 Navel-end opening

The navel-end opening is located on the stylar-end of the primary fruit. Its presence may be associated with the presence of the secondary fruit. Nonetheless, the size of the secondary fruit does not influence the size of the navel-end opening (Lima and Davies, 1984b). During fruit development the navel-end opening is usually noticeable from about six weeks after anthesis and reaches its final size at fruit maturity (Fig. 4) (Lima and Davies, 1984b). Borders of the navel-end opening and tissues inside the navel-end cavity are made up of the rind of both the primary and secondary fruit (Lima and Davies, 1984b). The navel-end opening can vary in size from 0 to 50 mm in diameter (Lima and Davies, 1984b).

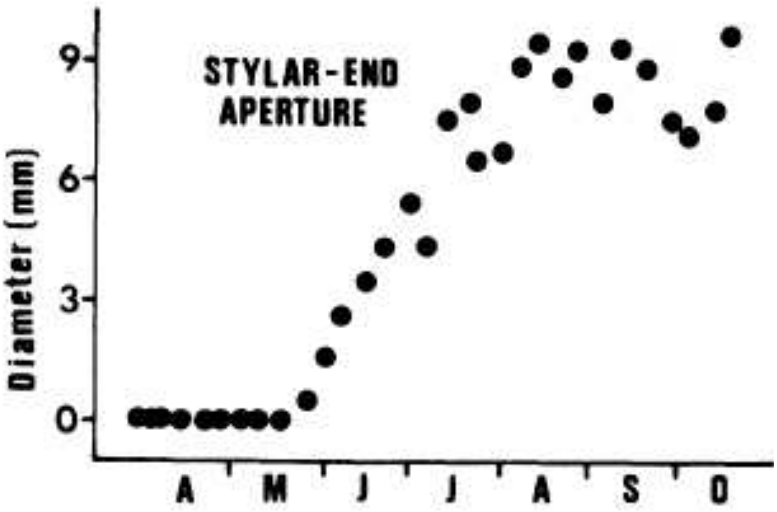

Fig. 4. Increase in the navel-end opening diameter of the navel orange from anthesis until fruit maturity in the (Lima and Davies, 1984b).

3. CAUSES OF THE PRESENCE OF THE SECONDARY FRUIT

The cause of the secondary fruit in navel oranges is not exactly known. One hypothesis claims the involvement of high auxin levels in secondary fruit development. Gustafson (1939) reported that navel oranges had higher auxins levels compared to other sweet orange varieties. Stewart and Parker (1947) reported that grapefruit sprayed with 75 or 225 $mg \cdot L^{-1}$ 2,4-D developed small rudimentary secondary fruit. Similarly, Valencia orange fruit that had been treated with 225 $mg \cdot L^{-1}$ 2,4-D developed small secondary fruit complete with juice vesicles (Stewart and Klotz, 1947). This evidence may support the claim that auxins or other associated plant growth hormones play a role in the development of the secondary fruit.

4. CAUSES OF THE FORMATION OF A LARGE NAVEL-END OPENING

Various factors have been documented as having an effect on the formation of the navel-end opening and its size. An understanding of the causes of the formation of large navel-ends will assist in research to reduce the size of the navel-end and reduce the problems that are associated with it such as fruit splitting and stylar-end decay (Wager, 1939).

4.1 Climate

The formation of the navel-end opening in navel oranges and the extent of its size can be related to the climate in which the fruit is grown. Soule and Grierson (1986) reported that navel oranges produced in Mediterranean type climates with cool winters generally have a smaller secondary fruit and navel-end opening than those produced in warm humid subtropical areas. Similarly large navel-end openings have been reported to be more common in climates with hot, dry summers than those with cool, wet summers (Wager, 1941). However, the exact contribution of climate to the size of the navel-end opening has not been established.

4.2 Climatic conditions after fruit set

The climatic conditions after fruit set (post-blossom period) has been associated with the formation of the navel-end opening (Grout, 1992; Wager, 1939). Navel oranges have two sets of stylar tissue (Wager, 1939). Extreme weather conditions like hot and windy days after fruit set damage the external stylar tissue and causes the inner stylar tissue to grow and swell with the subsequent formation of a longitudinal fissure in the outer stylar tissue (Wager, 1939). The fissure enlarges as the fruit increases in size and the inner ovary bulges, resulting in the formation of an irregular shaped navel-end opening at fruit maturity, sometimes with a protruding secondary fruit (Wager, 1939).

4.3 Abnormal water relations

Irregular water relations when the fruit are large (>40 mm), can cause the formation of open navel-ends. If a hot day occurs during this period, the trees will wilt slightly and moisture will be withdrawn from the fruit by the wilting leaves (Wager, 1939). After irrigation the water may be returned faster than the ability of the rind to stretch resulting in cracks appearing at the

navel-end (O'Connell, 2006; Wager, 1939). As the fruit grows, the cracks expand and start to open up resulting in an open navel-end (Wager, 1939). Navel-end openings formed under these conditions are usually irregular and star shaped (Fig. 5).

Fig. 5. Star shaped navel-end formed due to irregular water relations.

4.4 Bearing position of fruit on a tree

The bearing position of fruit on a tree also influences the size of the navel-end opening. Wager (1939) reported that exposed fruit in the top of the canopy developed more open navel-ends than bottom inside fruit. In addition, fruit from the top of the tree on the south side developed more open navel-ends compared to fruit from the bottom of the north side (NH) (Lima and Davies, 1984a).

4.5 Other causes

There are also several other factors suggested to play a role in the formation of an open navel-end. Insects such as American bollworm (*Helicoverpa armigera*), often attack young fruit and consume the style at the navel-end (Moore et al., 2007; Wager, 1939). As the fruit develops, a hole is formed at the navel-end which grows and has the appearance of a rose in mature fruit (Fig. 6) or a protruding navel-end (Moore et al., 2007; Wager, 1939). The weakness of the thin skin at the navel-end may be an inherent factor and such fruit may be more prone to crack

open at the stylar-end and have an open navel-end (Grout, 1992; Wager, 1939). The length of time during which the style is still attached to the fruit may influence the formation of an open navel-end (Verreynne, 2008). If the style abscises early during fruit development more open navel-ends are likely to be formed than if the style stays attached for a longer time. The effect of crop load on navel-end size has not been reported, but final fruit size at harvest has been reported to have no influence on the size of the navel-end opening (Verreynne, 2008).

Fig. 6. Open navel-end with the appearance of a rose, showing signs of bollworm damage.

5. NAVEL-END OPENING AND FRUIT PHYSIOLOGICAL DISORDERS

The morphology of the navel orange with a secondary fruit and the open navel-end, predisposes the fruit to certain physiological disorders of which the most common are fruit splitting and secondary fruit yellowing. These disorders may be linked to the size of the navel-end opening (Lima and Davies 1981, 1984c; Wager, 1939). Physiological disorders of navel oranges are some of the major causes of summer fruit drop (Lima and Davies 1981, 1984a; Lima et al., 1980).

5.1 Fruit splitting

Fruit splitting is one of the major physiological disorders of navel oranges and may in some cases cause severe yield loss due to fruit drop (Lima and Davies, 1984a, 1984b; Lima et al., 1980). Fruit splitting starts at the open navel-end (Lima and Davies, 1981). The incidence of

fruit splitting in navel oranges has been associated with the size of the navel-end opening; with split fruit usually having larger navel-end openings (Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939).

In addition to the presence of the open navel-end, fruit splitting can develop due to abnormal water relations in navel oranges (Lima et al., 1980). Lima and Davies (1981) reported that fruit splitting was more frequent during and following rainy days. Fruit absorb more water during this period and the navel-end opening has a thinner rind presenting a weak point on fruit where splitting will start developing (Wager, 1939).

Fruit splitting triggers abscission of the primary fruit by causing the premature production of ethylene (Lima and Davies, 1984a, 1984d). Split fruit also attract insects such as fruit fly (*Drosophila melanogaster*) and provide an entry point for pathogens causing fruit to become mouldy and rotten (Fig. 7) (Wager, 1939).

Fig. 7. Fruit splitting in navel oranges accompanied by fruit decay. Note the large open navel-end.

5.2 Secondary fruit yellowing

Secondary fruit yellowing is a major cause of summer fruit drop in navel oranges (Lima and Davies, 1984a). It is a physiological disorder caused by the abscission of the secondary fruit and is followed by the subsequent abscission of the primary fruit (Lima and Davies, 1984a, 1984d). The cause of secondary fruit abscission can be attributed to the interruption of phloem translocated leaf photosynthates and the accompanied increase in fruit ethylene production (Lima and Davies, 1984a, 1984d). Ethylene production induces fruit abscission by stimulating cellulase activity which breaks down the vascular bundles connecting the primary fruit to the secondary fruit (Lima and Davies, 1984d; Lima et al., 1980).

Anatomical studies have shown that in fruit affected by secondary fruit yellowing, an abscission zone is formed where cells are transformed into a gelatinous mass with no distinguishable cell walls and other cell structures (Lima et al., 1980). This gelatinous mass is noticeable as a separation between the central axis and the secondary fruit (Lima et al., 1980). Application of 2,4-D before or during initiation of secondary fruit yellowing decreases its severity by decreasing the ethylene levels at the stylar-end of the fruit (Lima and Davies, 1984d). Secondary fruit yellowing also initiates subsequent invasion of fruit by insects and fungal organisms causing decay (Lima and Davies, 1984d).

6. NAVEL-END OPENING AND DISEASES IN CITRUS

Navel-end rot, also known as stylar-end decay (SED), is one of the diseases that affect navel oranges and is caused by the fungus *Alternaria citri*. The size of the navel-end opening influences the development of navel-end rot in navel oranges with larger navel-ends more prone to the disease (Lima and Davies, 1984a; O'Connell, 2006; Wager, 1939). The pathogen gains entrance to the fruit through the open navel-end (Wager, 1941). The thin rind at the navel-end also makes it easier for pathogens to penetrate into the fruit (Lima et al., 1980). Navel-end rot is also known as "black-heart" as the affected fruit becomes black and rotten internally (O'Connell, 2006; Wager, 1941). The incidence of navel-end rot is more severe in seasons where there are more fruit with open navel-ends (O'Connell, 2006). Other diseases associated with larger navel-end openings are *Phytophthora* brown rot and blue-green molds (*Penicillium digitatum* and *P. italicum*) (O'Connell, 2006).

Fruit affected by navel-end rot is easy to spot on trees as it assumes a bright yellow colour which makes it easy to remove such fruit when practicing orchard sanitation (Wager, 1941). Navel-end rot has also been associated with a large summer fruit drop because it stimulates fruit abscission (Lima and Davies, 1984a; Lima et al., 1980; O'Connell, 2006). Affected fruit normally drops from the tree before harvest (O'Connell, 2006).

7. INSECT DAMAGE ASSSOCIATED WITH OPEN NAVEL-ENDS

The navel-end opening provides an entry point for insects into the fruit (Lima et al., 1980). Insect damage at the navel-end causes premature fruit drop as it stimulates the development of an abscission layer (Soule and Grierson, 1986). Citrus bud mite [*Aceria sheldoni* (Ewing)] enlarges the navel-end opening, sometimes causing it to protrude (Grout, 1992; Searle and Smith-Meyer, 1998). In addition, insects such as grain chinch bug (*Macchiademus diplopterus*) and citrus mealybug (*Planoccus citri*) may hide inside the open navel-end which makes insect control very difficult (Verreynne, 2008).

8. USES OF 2,4-D IN CITRUS PRODUCTION

The synthetic auxin 2,4-D is used to influence plant growth and development in citrus production all over the world. When used in the correct manner, it offers significant economic advantages to growers (Stover et al., 2000). Excessive rates, improper timings, untested surfactants and suboptimal environmental conditions can result in phytotoxicity, erratic results or greatly reduced yields (Stover et al., 2000). Some of the various uses of 2,4-D in citriculture are for example, extending harvest time (Coggins, 1981; Sarooshi, 1982), increasing fruit size (Anthony and Coggins, 1999; Guardiola, 1997), postharvest calyx retention (Cronjé et al., 2005; Singh et al., 1977) and more recently the reduction of the size of the navel-end opening in navel oranges (Gardiazabal, 2006; Saavedra, 2006; Verreynne, 2008).

8.1 Navel-end size reduction

Krezdorn (1969) reported that dipping flowers in a combination of 20 $mg \cdot L^{-1}$ 2,4-D and 250 $mg \cdot L^{-1}$ GA reduced the size of the navel-end. Recently, Gardiazabal (2006) and Saavedra (2006) reported that 2,4-D applied at full bloom reduce the size of the navel-end and increase the percentage of fruit with closed navel-ends. Gardiazabal (2006) reported that 20 $mg \cdot L^{-1}$

2,4-D applied on 'Lane Late' navels in Chile at full bloom resulted in 49% closed navels compared to 3% in the control and reduced the navel-end size to 4.8 mm compared to 12 mm in the control. Similarly, Saavedra (2006) reported that 20 $mg \cdot L^{-1}$ 2,4-D applied at full bloom on 'Lane Late' navels increased the percentage of closed navel-ends to 38.1% compared to 25.9% in the control and reduced the size of the navel-end to 6.8 mm compared to 8.6 mm in the control. The 2,4-D treatment also reduced the percentage of fruit with split navel-ends to 8.5% compared to 16.3% in the control.

Preliminary studies in South Africa showed that the application of 25 $mg \cdot L^{-1}$ 2,4-D at 100% petal drop increased the percentage of closed navel-ends and reduced the average navel-end size in 'Palmer', 'Robyn' and 'Lane Late' navel orange (Verreynne, 2008). The percentage of closed navel-ends was increased by 30% in 'Palmer' navel, 24% in 'Robyn' navel and 39% in 'Lane Late' navel (Verreynne, 2008). The mode of action of 2,4-D in closing the navel-end appears to be related to the delay in style abscission (Verreynne, 2008).

The application of 2,4-D for navel-end reduction had no significant effect on fruit size and fruit shape (Gardiazabal, 2006; Saavedra 2006). It reduced juice percentage and titratable acidity and increased the soluble solids to acid ratio (Saavedra, 2006). The total soluble solids and rind thickness were not affected (Saavedra 2006; Verreynne, 2008). Treated fruit also had greener navel-ends compared to the control fruit (Verreynne, 2008). The styles persisted on treated fruit until late into fruit development (Krezdorn, 1969; Verreynne, 2008) and there was no effect on yield or the number of fruit per tree (Gardiazabal, 2006).

8.2 Fruit size control

Fruit size is one of the most important parameters of external fruit quality and its importance has increased markedly in recent times (Guardiola, 1997; Guardiola and Garcia-Luis, 2000). Larger fruit realize better returns for the grower making it more commercially viable to have larger sized fruit, as consumers are willing to pay a premium price for it (El-Otmani et al., 1996). In some markets, regulations have been adopted to stipulate the acceptable minimum fruit size (Guardiola, 1997). Therefore, small fruit cannot be exported to these markets and earnings from these small fruit are often lower than the cost of production (Erner et al., 1993; Guardiola, 1997).

Stewart and Klotz (1947) suggested the possible use of 2,4-D to increase fruit size of Valencia and 'Washington' navel oranges. Applications of 2,4-D caused an increase in fruit size which was proportional to the concentration of 2,4-D applied. Similarly, 2,4-D applied at full bloom increased the fruit size of 'Washington' navel orange (Stewart et al.,1951a).

8.2.1 Mode of action

The application of 2,4-D to increase fruit size may act in two ways depending on the timing of the application. If 2,4-D is applied during physiological fruit drop, it acts as a thinning agent, reducing competition for carbohydrates amongst developing fruitlets (Agusti et al., 2002; Guardiola, 1997). Alternatively, when applied at full bloom, 2,4-D increases the sink strength of the fruit by acting as a fruit growth enhancer without the thinning effect (Agusti et al., 2002; Guardiola, 1997). This allows for increased carbohydrate accumulation within the fruit resulting in an increase in fruit size (Guardiola, 1997). The possible modes of action of auxins and how they affect final fruit size is presented in Fig. 8.

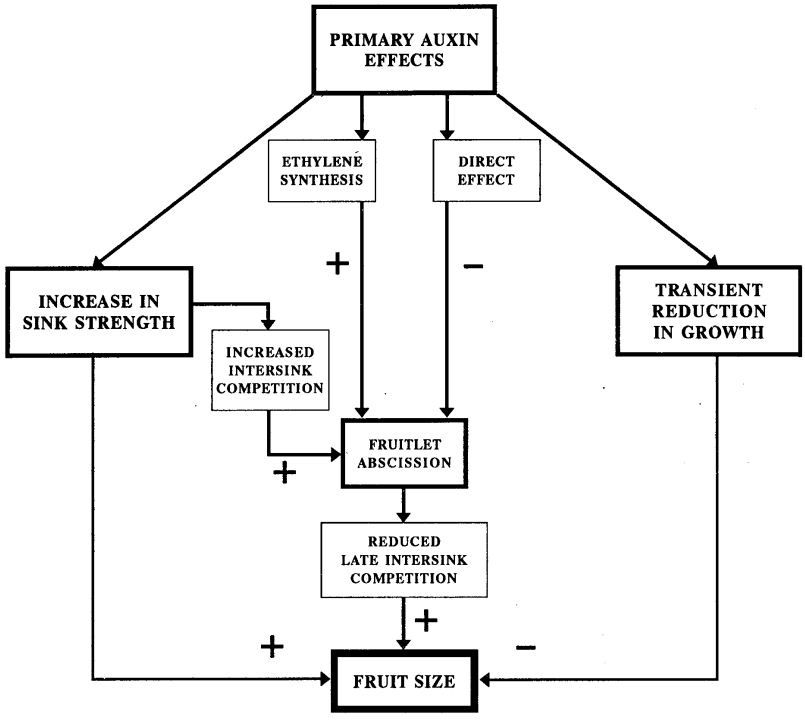

Fig. 8. Diagram showing the effects of synthetic auxins on final fruit size (Guardiola, 1988).

In some countries, 2,4-D is used as a thinning agent of citrus (Agusti et al., 2002). It acts by inducing ethylene biosynthesis thereby resulting in fruitlet abscission (Guardiola, 1997). The

2,4-D must be applied before the end of physiological fruit drop as later applications are not effective (El-Otmani et al., 2000; Guardiola, 1997). By thinning the fruit, it reduces competition for assimilates amongst the young fruitlets resulting in increased fruit size of the remaining fruit.

As a fruit growth enhancer, 2,4-D acts by increasing the capacity of the developing fruit to act as sinks for water and assimilates (El-Otmani et al., 2000). Stewart and Klotz (1947) was first to suggest that increased fruit size was a direct response to the 2,4-D itself and not an indirect response of fruit thinning. Stewart et al. (1951a) reported that as little as 4 $mg \cdot L^{-1}$ of 2,4-D at full bloom increases fruit size in 'Washington' navels. It was concluded that 2,4-D had a direct effect on the growth of tissues as an increased fruit size was obtained with no reduction in the number of fruit per tree. The application of 17 to 20 $mg \cdot L^{-1}$ of 2,4-D at full bloom also increased fruit size in 'Esbal' Clementine without any fruit thinning effect (Guardiola and Garcia-Luis, 2000). The addition of 5% potassium nitrate to the spray solution increased the effectivity of the 2,4-D (Guardiola and Garcia-Luis, 2000). It is important to note that not all cultivars respond to auxins as fruit growth enhancers (Guardiola, 1997).

8.2.2 Effect on external fruit quality

The application of 2,4-D to increase fruit size by fruit thinning has some negative effects on external fruit quality, usually observed at high concentrations (above 75 $mg \cdot L^{-1}$). Stewart and Klotz (1947) reported that the application of 225 $mg \cdot L^{-1}$ 2,4-D on 'Valencia' and 'Washington' navel oranges resulted in a coarse rind due to enlarged oil glands. The fruit were also cylindrical in shape and the Valencia oranges developed a small secondary fruit. Similar effects were noted on grapefruit (Stewart and Parker, 1947). When applied as a fruit growth enhancer, treated fruit were greener and more elongated compared to the control fruit (Stewart et al., 1951a).

8.2.3 Effect on internal fruit quality

The application of 2,4-D for fruit thinning has several effects on internal fruit quality. It increased the percentage of the rind as well as the rag and reduced the juice content of the treated fruit (Stewart and Klotz, 1947). There was also a slight decrease in titratable acids and an increase in the soluble solids to acid ratio (Stewart and Klotz, 1947). In 'Washington' navel orange there was the development of small rudimentary seeds in treated fruit (Stewart

and Klotz, 1947). When applied at full bloom to act as a growth enhancer, 2,4-D also increased the percentage of the rind as well as the rag and reduced the juice percentage in treated fruit (Stewart et al., 1951a). In addition, the total soluble solids were reduced and the titratable acid content was higher in juice from treated fruit (Stewart et al., 1951a).

8.2.4 Effect on vegetative growth

Foliar applications of 2,4-D damages young leaves on new growth flushes. When 2,4-D is applied as a fruit thinner during physiological fruit drop, no new growth flushes are present and leaf damage is minimal at low concentrations (25 $mg \cdot L^{-1}$ and below) (Stewart and Klotz, 1947), but high concentrations e.g. 225 $mg \cdot L^{-1}$ damage the mature leaves resulting in irregular chlorotic areas which last for a few months (Stewart and Klotz, 1947; Stewart and Parker, 1947).

The application of 2,4-D at full bloom to act as a growth enhancer, damaged the young leaves on the new growth flushes (Stewart and Klotz, 1947; Stewart et al., 1951a). The damage to young leaves was more pronounced at high concentrations and decreased with decreasing concentration of 2,4-D until barely noticeable at 5 $mg \cdot L^{-1}$ (Stewart and Klotz, 1947). At harvest the damage was not noticeable suggesting that the leaf damage was temporal or that the damaged leaves dropped (Stewart and Klotz, 1947). The leaf curling did not reduce yield or affect fruit quality (Stewart et al., 1951a). This may be explained by the fact that citrus trees produce several leaf growth flushes and the damage done to one flush may be compensated for by the succeeding flushes (Stewart et al., 1951a).

8.2.5 Effect on flower retention

Treatment with 2,4-D at full bloom, to increase fruit size increased the number of fruitlets retained on the tree at petal drop (Stewart and Klotz, 1947). The flower retention effect lasted for 8 weeks after application (Stewart and Klotz, 1947). Decreased flower abscission was evident on treated trees with the ovaries, stamens and corolla in nearly all flowers remaining securely attached to the receptacle compared to the control (Stewart and Klotz, 1947).

8.2.6 Effect on ovaries and fruit abscission

The application of 2,4-D as a fruit growth enhancer affected ovaries and the initial development of fruit abscission. Ovaries of treated trees were pale yellow in colour, unlike the usual deep green colour (Stewart and Klotz, 1947). Initial fruitlet abscission was decreased on treated trees for 12 weeks after application, however after that abscission in treated trees was similar to the control (Stewart and Klotz 1947; Stewart et al., 1951a). In some cases, 2,4-D reduced the fruit number, but yield was not affected due to an increased fruit size (Stewart et al., 1951a).

8.3 Prolonging of harvest time (late hang)

Fruit drop can cause severe loss of yield, especially during the later part of the harvest season and 2,4-D is commonly used in citrus production to reduce fruit drop associated with delayed harvesting (Coggins and Hield, 1968). It is effective in reducing fruit drop of Valencia oranges (Stewart and Klotz, 1947; Stewart et al., 1952), navel oranges (Stewart et al., 1951a) and grapefruit (Stewart and Parker, 1947, 1954).

Depending on the intended harvest date 2,4-D is applied after fruit has matured. The amine salt or the isopropyl ester of 2,4-D is applied at 20 $mg \cdot L^{-1}$ in the United States of America (Coggins, 1981), and in South Africa the amine formulation is at 10 to 20 $mg \cdot L^{-1}$ applied with 10 $mg \cdot L^{-1}$ GA_3 (El-Otmani et al., 2000). Fruit drop is reduced by 2,4-D whilst GA_3 reduces both pre-harvest and post-harvest rind disorders by strengthening the rind and delaying senescence (Coggins et al., 1984; El-Otmani et al., 2000).

8.3.1 Mode of action

Drop of mature fruit is caused by changes in the cellular walls of the abscission zone mainly at the peduncle (Stewart and Hield, 1950). Monselise and Goren (1978) reported that the application of 2,4-D prevented the dropping of fruit by maintaining the cells at the abscission zone, preventing the synthesis of hydrolytic enzymes such as cellulase, which degrade the cell walls. The application of 2,4-D is also thought to have an effect on vascular growth in the abscission zone by changing the physiology of vascular elements, particularity the phloem (Stewart et al., 1951a). As the vascular connections to the fruit remain unbroken for a longer time, fruit drop is thereby delayed (Coggins et al., 1984).

8.3.2 Effect on fruit quality

The application of 2,4-D to prolong harvest did not affect the juice percentage, titratable acidity and the soluble solid content (Stewart and Klotz, 1947; Stewart and Parker, 1947; Stewart et al., 1951a). Externally, treated fruit (only 2,4-D) were firmer, showing characteristics of early season fruit, with no signs of aging (Stewart et al., 1951a).

8.3.3 Leaf damage

The application of 2,4-D damages young growth flushes in citrus (See section 8.2.4). The damage is caused by the epinastic reaction of the young leaves to 2,4-D which manifests as downward bending of leaves at the margins (Salisbury and Ross, 1992). The application of 2,4-D to prolong harvest time is recommended in between the growth flushes so as to cause minimal leaf damage (Stewart and Klotz, 1947; Stewart et al., 1951a). The ester formulations of 2,4-D are more volatile than the amine formulations and cause more leaf damage (Monaco et al., 2002). Within the ester group itself, short chain esters such as isopropyl esters are more volatile than long chain esters such as iso-octyl esters (Coggins and Hield, 1968; Gile, 1983; Monaco et al., 2002). In citriculture the ester formulations can be used since applications are done at low concentrations while less volatile formulations (e.g. amines) can be used at high concentrations (Monselise, 1979).

8.4 Postharvest calyx retention

Citrus fruit are harvested by cutting the stem close to the calyx (outermost floral whorl consisting of 5 sepals), leaving behind a piece of the pedicel. The piece of pedicel and the calyx is commonly referred to as the button. In freshly harvested fruit the calyx is green, but as the fruit are stored the button turns brown and dies off. The button may fall off leaving behind a point where pathogens can enter. *Alternaria* rot is one of the diseases that may develop and is characterized by a blackened button and eventual rot of the whole fruit (Stewart et al., 1951b).

8.4.1 Mode of action

The postharvest application of 2,4-D reduces the incidence of *Alternaria* rot by retarding the abscission of the button (Cronjé et al., 2005; Singh et al., 1977; Stewart et al., 1951b). By

delaying the loss of chlorophyll from the calyx, 2,4-D keeps it intact for a longer time thereby blocking the entrance of pathogens (Coggins, 1986; Smilanick et al., 2006; Singh et al., 1977). Application is done in the packhouse as a dip treatment at a rate of 500 $mg \cdot L^{-1}$ (Cronjé et al., 2005; Singh et al., 1977).

8.4.2 Effect on fruit quality

The postharvest application of 2,4-D increased the storage life of fruit and delayed the development of the yellow colour in the rind during storage (El-Otmani et al. 1990; Stewart et al., 1951b).

8.5 Other reported uses of 2,4-D in Citriculture

Other potential uses of 2,4-D in citriculture have been reported. The application of 2,4-D reduced secondary fruit yellowing (Lima and Davies, 1981,1984d) possibly through the inhibition of secondary fruit abscission (Lima and Davies, 1984d). Fruit splitting in 'Washington' navel oranges (Coggins and Hield, 1968) and 'Nova' mandarin (Almela et al., 1994; Garcia-Luis et al., 2001; Greenberg at al., 2006) was reduced by the application of 2,4-D (See Paper 2). In Valencia oranges, the onset of granulation was reduced by the application of 2,4-D to small developing fruits (Coggins and Hield, 1968). The severity of summer fruit drop can be reduced by the application of 2,4-D (Lima and Davies,1981, 1984c). Leaf abscission caused by Ethrel which is used as a fruit abscission agent to aid mechanical harvesting of fruit can be decreased by adding 2,4-D to the spray mixture (Bondad, 1976; Ismail, 1970).

8.6 Combination of 2,4-D with other sprays

To save resources, growers may need to apply two or more chemicals at the same time. As 2,4-D is compatible with many nutritional and pesticidal chemical sprays, the grower can include it in an already existing spray program (Coggins and Hield, 1968; Stewart et al., 1951a). In fact, 2,4-D may in some cases counteract the negative effects of chemical sprays that it is mixed with. Leaf and fruit drop caused by pesticidal oil sprays can be reduced by the addition of 2,4-D to the spray mixture (Stewart and Ebeling, 1946; Wessels and Holtzhausen, 1984).

8.7 Herbicide

When 2,4-D was initially developed during World War II, its primary use was for its herbicidal properties. It is very effective when used in grass cereal crops (monocots) as it only kills dicots allowing for very effective broadleaf weed control. For example in wheat it is applied at concentrations of 3500 to 6000 $mg \cdot L^{-1}$ with 150 to 200 l of spray solution used per hectare (Dow AgroSciences, 2007; Pieterse, 2009). It is thought to change many enzymatic and non-enzymatic process in the plant, thereby disturbing the hormone balance and protein synthesis in the plant (Duke, 1990; Mitchell, 1961). The combined effect of all these processes results in the eventual death of the plant. Some of the noted effects include an increase in the moisture content of tissues affected, hydrolysis of reserve carbohydrates, depletion of sugars and proliferation of responsive parenchymous cells (Mitchell, 1961). It concentrates in young embryonic or meristematic tissues that are growing rapidly and affects these tissues more than the established tissues (Ashton and Monaco, 1991). If concentrations are not high enough to cause direct death, the damage done to the vascular tissues will eventually result in a slower death as nutrients supplied via these tissues are gradually cut off (Ashton and Crafts, 1973).

9. EFFECT OF 2,4-D ON PHOTOSYNTHESIS

Freeland (1949) measured photosynthesis by the gas exchange method and reported that 2,4-D applied at 100 $mg \cdot L^{-1}$ inhibited photosynthesis in bean leaves by about 20%. In citrus, Wedding et al. (1954) studied the effect of 2,4-D on photosynthesis of detached 'Washington' navel orange leaves and reported that 2,4-D inhibited photosynthesis. The rate of photosynthesis was inversely proportional to the logarithm of the concentration of 2,4-D molecules and it was speculated that 2,4-D had an effect on some rate limiting enzyme involved in photosynthesis (Wedding et al., 1954).

10. CONCLUSION

Navel oranges are a unique type of the sweet orange with a secondary fruit and a resultant navel-end opening. The presence of the opening at the navel-end adds a unique set of problems to the production of navel oranges. Physiological disorders like fruit splitting resulting in fruit drop can be associated with the open navel-end. The navel-end opening also provides a harboring place for insects making it difficult to control them. In addition,

pathogens gain access to the fruit through the open navel-end. Navel oranges are also culled, depending on the size of the navel-end opening. The control of the size of the navel-end opening is therefore necessary in the production of navel oranges.

The main objective of the study is to determine the effect of 2,4-D on the navel-end opening of navel oranges. The best timing of application and the optimum concentration to be used at that timing with minimal negative effects on fruit quality will be determined. Other objectives of the study are to determine the effect of 2,4-D application on fruit quality, yield, physiological disorders, fruit set and post-harvest storage quality of the fruit. The photosynthetic ability of the damaged leaves will also be evaluated. The growth and development of the primary and secondary fruit will be followed through the season to elucidate their influence on the development of the navel-end opening. In addition, 2,4-D will be applied to a split prone mandarin cultivar to evaluate its efficacy on fruit splitting.

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PAPER 1: EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON THE NAVEL-END OPENING IN NAVEL ORANGES [*CITRUS SINENSIS* (L.) OSBECK]

Abstract

The size of the navel-end opening is an important parameter for external fruit quality in navel oranges. Fruit with large open navel-ends are predisposed to splitting and navel-end rot in the orchard and are culled in the packhouse, thereby reducing the export packout. The application of 2,4-D to increase the percentage of closed navel-ends and reduce the size of the navel-end opening was evaluated over two consecutive seasons, 2007/2008 and 2008/2009. Studies were carried out on six different navel orange cultivars, namely 'Washington', 'Newhall', 'Navelina', 'Palmer', 'Autumn Gold' and 'Robyn' in four different production areas, namely Citrusdal, Clanwilliam, Heidelberg and Addo, South Africa. Treatments were applied at full bloom (FB), 100% petal drop (PD), 2 weeks after 100% petal drop (2 WAPD) and 4 weeks after 100% petal drop (4 WAPD), at 15 mg·L^{-1} to 45 mg·L^{-1} of 2,4-D, as the iso-octyl ester to determine the most effective timing and concentration. The application of 2,4-D at FB increased the percentage of closed navel-ends (by up to 42%) and reduced the average navel-end size of all the fruit sampled (by up to 5 mm), in all the cultivars and the different production regions, over both seasons, regardless of the concentration applied. The average navel-end size of only the fruit with open navel-ends was not affected, therefore 2,4-D seems to close the navel-end opening completely, rather than making it smaller. Late applications at PD, 2 WAPD and 4 WAPD were generally ineffective. The yield and total fruit number per tree were not affected by the treatments. There were no major negative side effects on external and internal fruit quality except for the reduction in juice content (%) especially with the PD and later applications. The postharvest storage quality of the fruit was not affected by the treatments. The application of 2,4-D damaged the young leaves on new growth flushes, but had no effect on their photosynthetic capacity. Stylar abscission was delayed by the FB application of 2,4-D, which most likely plays a role in the mode of action by which 2,4-D keeps the navel-ends closed. The application of 15 mg·L^{-1} 2,4-D at FB can be used to increase the percentage of fruit with closed navel-ends and thereby increase export packouts. Furthermore, 2,4-D may reduce fruit splitting and navel-end rot.

Keywords: *attached styles; closed navel-ends; fruit splitting; full bloom (FB); leaf damage; navel-end rot.*

Introduction

The size of the navel-end opening is one of the parameters that are evaluated for external fruit quality in navel oranges. Fruit with large open navel-ends are culled in the packhouse which reduces the export packout (Verreynne, 2008). The maximum acceptable navel-end opening diameter for export fruit is 20 mm (Grout, 1992). The presence of large navel-end openings also causes certain problems such as the higher incidence of stylar-end decay in fruit with large navel-end openings (Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939, 1941). Physiological disorders such as fruit splitting are more common in fruit with large navel-end openings (Krezdorn, 1969; Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939) and large navel-end openings also provide an entry point and harboring place for insects making it difficult to control them (Soule and Grierson, 1986). Some of the factors that influence the size of the navel-end opening are, the weather after fruit set (Grout; 1992; Wager, 1939), abnormal water relations (O'Connell, 2006; Wager 1939), the bearing position of fruit (Lima and Davies, 1984a; Wager 1939) and insect damage (Moore et al., 2007; Wager 1939).

The synthetic auxin 2,4-D is used as a plant growth regulator to influence plant growth and development in citrus production by manipulating key physiological processes both in the orchard and the packhouse (Lovatt, 2005; Stover et al., 2000; Wright, 2004). The main commercial uses of 2,4-D in citrus production are: to increase fruit size (Anthony and Coggins, 1999; Guardiola, 1997), prolong harvest time (Coggins, 1981; Sarooshi, 1982) and postharvest calyx retention (Cronjé et al., 2005; Singh et al., 1977; Wright, 2004). It is used at low concentrations thereby posing low risk to both man and the environment whilst leaving no hazardous residues (El-Otmani et al., 2000, Monselise, 1979).

The reduction in the size of the navel-end opening by 2,4-D was first reported by Krezdorn (1969) who showed that dipping flowers in a combination of 20 $mg \cdot L^{-1}$ 2,4-D and 250 $mg \cdot L^{-1}$ GA reduced the size of the navel-end opening. Recently Gardiazabal (2006) and Saavedra (2006) reported that 2,4-D applied at full bloom reduced the size of the navel-end opening of navel oranges and increased the percentage of fruit with closed navel-ends. Gardiazabal (2006) reported that 20 $mg \cdot L^{-1}$ 2,4-D applied on 'Lane Late' navels in Chile at full bloom resulted in 49% closed navels compared to 3% in the control and reduced the navel-end size to 4.8 mm compared to 12 mm in the control. Similarly, Saavedra (2006) reported that 20 $mg \cdot L^{-1}$ 2,4-D applied at full bloom on 'Lane Late' navels increased the percentage of closed navel-ends to 38.1% compared to 25.9% in the control and reduced the size of the navel-end

to 6.8 mm compared to 8.6 mm in the control. Application of 2,4-D also reduced the percentage of fruit with split navel-ends (Saavedra, 2006).

Preliminary studies in South Africa showed that the application of 25 $mg \cdot L^{-1}$ 2,4-D at 100% petal drop increased the percentage of closed navel-ends and reduced the average navel-end size in 'Palmer', 'Robyn' and 'Lane Late' navel oranges (Verreynne, 2008). The application of 2,4-D increased the percentage of closed navel-ends by 30% in 'Palmer' navel, 24% in 'Robyn' navel and 39% in 'Lane Late' navel (Verreynne, 2008). The mode of action appears to be related to the delay in style abscission, thereby keeping the navel-end closed (Verreynne, 2008).

The reduction in the size of the navel-end opening would bring several advantages to the grower such as higher export packouts, more effective insect control and a reduction in both fruit splitting and stylar-end decay (Verreynne, 2008). The main objective of the study was to determine the effect of different timings and concentrations of 2,4-D on the navel-end opening on different cultivars in different production areas in South Africa. Further objectives of the study were to determine the effect of 2,4-D application on fruit quality, fruit set, yield, physiological rind disorders, post-harvest storage quality of the fruit and to assess the photosynthetic ability of leaves damaged by 2,4-D application. The efficacy of a different formulation of 2,4-D was also evaluated.

Materials and Methods

Plant material and treatments

'Washington' navel orange. The study was conducted on 'Washington' navel orange trees grafted on Rough lemon rootstock in Citrusdal (32°30'S 19°E), South Africa. The orchard was planted in 1984 with a tree spacing of 6 m between rows and 6 m within rows and with an east-west row direction. Treatments included an untreated control, 2,4-D applied at 15 $mg \cdot L^{-1}$, 25 $mg \cdot L^{-1}$ and 35 $mg \cdot L^{-1}$ at both full bloom (FB) or 100% petal drop (PD).

'Newhall' navel orange. The study was conducted on 'Newhall' navel orange trees grafted on Rough lemon rootstock in Citrusdal (32°30'S 19°E), South Africa. The orchard was planted in 1993 with a tree spacing of 5 m between rows and 2 m within rows and with a north-south row direction. Treatments included an untreated control, 2,4-D applied at 25 $mg \cdot L^{-1}$ at FB or

at PD, 25 mg·L^{-1} + 10 mg·L^{-1} gibberellic acid (GA) (Progibb ™) at PD , 25 mg·L^{-1} at two weeks after petal drop (2 WAPD) and 25 mg·L^{-1} at four weeks after petal drop (4 WAPD). GA was included to improve fruit set.

'Navelina' navel orange. The study was conducted on 'Navelina' navel orange trees grafted on Rough lemon rootstock in Citrusdal (32°30'S 19°E), South Africa. The orchard was planted in 1993 with a tree spacing of 5 m between rows and 2 m within rows and with a north-south row direction. Treatments included an untreated control, 2,4-D applied at 15 mg·L^{-1}, 25 mg·L^{-1}, 25 mg·L^{-1} + 10 mg·L^{-1} GA, 35 mg·L^{-1} and 45 mg·L^{-1} at PD.

'Palmer' navel orange. The study was conducted on 'Palmer' navel orange trees grafted on Rough lemon rootstock in Addo (33°26'S 25°44'E), South Africa. The orchard was planted in 2001 with a tree spacing of 6 m between rows and 4 m within rows and with a north-south row direction. Treatments included an untreated control, 2,4-D applied at PD at 15 mg·L^{-1}, 20 mg·L^{-1}, 25 mg·L^{-1} and 30 mg·L^{-1}.

'Autumn Gold' navel orange. The study was conducted on 'Autumn Gold' navel orange trees grafted on Carrizo citrange rootstock in Heidelberg (34°06'S 20°57'E), South Africa. The orchard was planted in 1999 with a tree spacing of 5 m between rows and 2 m within rows and with an east-west row direction. Treatments included an untreated control, 2,4-D applied at 15 mg·L^{-1} (ester) at FB, 25 mg·L^{-1} (ester) at FB, 25 mg·L^{-1} (amine) at FB, 35 mg·L^{-1} (ester) at FB and 25 mg·L^{-1} (ester) at PD.

'Robyn' navel orange. The study was conducted on 'Robyn' navel orange trees grafted on Rough lemon rootstock in Clanwilliam (32°20'S 18°50'E) South Africa. The orchard was planted in 1987 with a tree spacing of 6 m between rows and 4 m within rows and with a north-south row direction. Treatments included an untreated control, 2,4-D applied at 20 mg·L^{-1} or 25 mg·L^{-1} at FB, 20 mg·L^{-1} or 25 mg·L^{-1} at PD, 25 mg·L^{-1} at 2 WAPD and 25 mg·L^{-1} at 4 WAPD.

Experimental Design

In all the experiments, each treatment consisted of eight single tree replicates in a randomized complete block design with buffer trees between treated trees. Trees were chosen for uniformity in size and only healthy trees were used. All experiments were carried out in

commercial orchards under standard production practices. Trials were conducted in the growing seasons of 2007/2008 and 2008/2009 for 'Washington', 'Newhall', 'Navelina' and 'Palmer' navel orange. For 'Autumn Gold' and 'Robyn' navel orange, only one trial was conducted in the 2008/2009 and 2007/2008 seasons, respectively.

Spray material and application method

Only 2,4-D ester (iso-octyl) was used in all experiments except for 'Autumn Gold' navel orange where an additional 2,4-D amine (dimethylamine salt) treatment was applied. A non-ionic wetting agent (Break-Thru®) with the active ingredient polyether-polymethylsiloxane-copolymer (1000 $g \cdot L^{-1}$) was added to the spray solution at a rate of 5 ml per 100 L of spray solution in all the experiments. Applications were made using a hand gun sprayer until run-off.

Data collection and evaluations

At commercial harvest, a full lug box (average 80 fruit) was collected from all sectors of each replicate. Fruit diameter and navel-end size was measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) on site. The average navel-end size (all fruit) was calculated for all the fruit sampled in the lug box. Fruit with closed navel-ends were then removed from sample and the average navel-end size of only the fruit with open navel-ends calculated. Two representative sub samples of 12 fruit each were taken from the lug box for further analysis.

Yield (kg) was determined per tree replicate for 'Newhall', 'Navelina' and 'Robyn' navels by harvesting all the fruit and recording the weight on an electronic scale (W22 Series, UWE Co, Hsin Tien, Taiwan). Yield was not measured for 'Palmer', 'Autumn Gold' and 'Washington' navels as it was not practical.

On the first sub sample of 12 fruit, the following evaluations were done. Fruit rind colour was determined based on the no. 34 CRI colour chart for oranges [Citrus Research International (CRI), 2004; Appendix 1], with 8 being dark green and 1 a fully developed orange colour. Navel-end colour was evaluated on a scale of 0 to 4 with 4 being a dark green navel-end and 0 a fully coloured navel-end (Appendix 2). Fruit was scored for creasing severity by dividing the fruit into four equal segments, with 0 if there was no creasing and 4 if all segments of the fruit were creased. Creasing incidence (%) was calculated by dividing the number of creased

fruit by the total number of fruit evaluated. Fruit diameter, fruit height and pedicel diameter were measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). Fruit shape was determined by calculating a ratio of fruit diameter: fruit height.

Fruit were cut into half along the equatorial plane for internal quality determinations. Rind thickness at the sides of the fruit and the diameter of the central axis (open pithy core in the middle of the fruit) were measured for each fruit using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). Fruit were also scored for any visible symptoms of granulation. The fruit were then juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth and the juice percentage was determined by dividing the weight of the juice by the total fruit weight. ^{o}Brix from the extracted juice was determined using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan). Titratable acidity (TA) expressed as citric acid content was determined by titrating 20 ml of the extracted juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator. The ^{o}Brix:TA ratio was calculated by dividing the ^{o}Brix values by the TA values.

On the second sub sample, 6 fruit were used for post-harvest storage evaluation and 3 fruit for peelability studies for the 2007/2008 season for all the trials. For the 2008/2009 season postharvest evaluation was only done for 'Washington' and 'Palmer' navels since no differences had been found in the 2007/2008 season. Peelabilty studies were not done in the 2008/2009 season for the same reason.

'Washington', 'Newhall', 'Navelina' and 'Robyn' navel fruit were stored at -0.5°C for four weeks whilst the 'Palmer' navel fruit were stored at 4.5°C for the same time period. After four weeks fruit was taken out of the cold room and scored for chilling injury, stem end rind breakdown, post-harvest pitting and the condition of the calyx. Fruit was then left at room temperature for one week to simulate shelf life after which it was re-evaluated for the same parameters.

Peelability studies were done using a Citripeel® citrus peeler. Fruit was scored for ease of peeling on a scale of 1 to 4 with 1 being the easiest to peel and 4 the most difficult to peel (Appendix 3).

The gas exchange method was used to measure photosynthesis rate of young 'Navelina' navel orange leaves in terms of net CO_2 uptake using an infra-red gas analyser (IRGA) (LI-6400

Portable Photosynthesis System, LI-COR, Lincoln Nebraska, USA). Measurements were done on leaves from the spring flush on the untreated control and those treated with 45 $mg \cdot L^{-1}$ 2,4-D at PD. Spots (immediate measurement of the gas exchange system of the plant) were done on two leaves per tree replicate. These spot readings gave an estimation of the leaf photosynthesis capacity ($\mu mol.m^{-2}.s^{-1}$) in terms of rate of net CO_2 uptake. The variables measured were A_{max} (light saturated rate of net CO_2 uptake), A_{sat} (light and CO_2 saturated rate of net CO_2 uptake) and R_d (dark respiration rate). Spots were done at ambient temperature and total leaf area per spot was 6 cm^2. Air flow in the cuvette was kept constant for all spots at 200 μmol.

At harvest, samples of fruit from the 'Navelina' navel orange treated with 45 $mg \cdot L^{-1}$ 2,4-D at PD were sent for residue analysis. Samples were analysed in accordance with the analytical method LCMS at Hearshaw and Kinnes Analytical Laboratory, Tokai, Cape Town.

Fruit set was evaluated by tagging five flowering shoots per tree and the number of flowers were counted at spraying and two weeks after spraying. Fruit set (%) was calculated by dividing the number of fruit that set by the original number of flowers.

Statistical analysis

Statistical analysis of variance (ANOVA) was carried out using PROC GLM (version 9.1, SAS Institute Inc., Cary, NC, USA). Mean separation was conducted by least significant difference (LSD) where applicable ($P = 0.05$) and appropriate contrasts where carried out.

Results

'Washington' navel orange

2007/2008: Fruit diameter at harvest (2008/06/23) was not affected by the application of 2,4-D (Table 1). The application of 2,4-D at FB (all concentrations) significantly reduced the average navel-end size (all fruit) ($P = 0.0071$) with no trend between the different concentrations applied, whilst all the PD applications had no effect. There was no difference in navel-end size of only fruit with open navel-ends between the treated and the control fruit. The application of 2,4-D at FB significantly increased the percentage of closed navel-ends at all the concentrations applied ($P = 0.0005$), while all the PD applications had no effect.

There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 2). The fruit shape, the central axis diameter and the pedicel diameter were not affected by 2,4-D treatment (Table 3). Although 25 $mg \cdot L^{-1}$ 2,4-D at FB significantly increased the rind thickness, no clear trend was observed due to the 2,4-D application. There were no significant differences in the ^{o}Brix, the TA, the ^{o}Brix:TA ratio and the juice content (%) between the treated and the control fruit (Table 4). In addition, granulation was not visible in any of the fruit sampled. The application of 2,4-D at FB at all concentrations significantly increased the initial fruit set ($P = 0.0005$) (Table 5).

2008/2009: Fruit diameter at harvest (2009/06/08) was not affected by the application of 2,4-D (Table 6). The application of 2,4-D significantly reduced the average navel-end size (all fruit) in all treatments except at 15 $mg \cdot L^{-1}$ at PD and also reduced the average navel-end size of only fruit with open navel-ends in all treatments except at 15 $mg \cdot L^{-1}$ and 25 $mg \cdot L^{-1}$ at PD. A linear reduction in the average navel-end size (all fruit) was observed with an increasing 2,4-D concentration ($P = 0.0169$). The FB applications gave better results than the PD applications in reducing the average navel-end size (all fruit). The application of 2,4-D at FB at all concentrations significantly increased the percentage of closed navel-ends ($P = 0.0013$) with the PD applications having no effect. The FB applications gave better results than the PD applications in increasing the percentage of closed navel-ends.

The fruit rind colour and the colour at the navel-end were not affected by the 2,4-D applications (Table 7). All the treatments except 15 $mg \cdot L^{-1}$ and 25 $mg \cdot L^{-1}$ at FB significantly reduced creasing severity ($P = 0.0002$). Although all the treatments reduced the creasing incidence, the reduction was not always significant. Fruit shape was not affected by the application of 2,4-D (Table 8). All the 2,4-D treatments except at 15 $mg \cdot L^{-1}$ at FB significantly increased the central axis diameter ($P = 0.0002$). There was no clear trend in rind thickness due to 2,4-D treatment at FB, but all the PD applications significantly reduced rind thickness compared to the control. Only 35 $mg \cdot L^{-1}$ 2,4-D applied at PD significantly increased the pedicel diameter ($P = 0.0001$).

The ^{o}Brix, the TA and the ^{o}Brix:TA ratio were not affected by the application of 2,4-D (Table 9). Juice content (%) was significantly increased by 15 $mg \cdot L^{-1}$ at FB or PD whilst 35 $mg \cdot L^{-1}$ 2,4-D applied at PD significantly reduced the juice content (%). The PD applications had a

significantly lower juice content (%) than FB applications (P = 0.0049), whilst a linear reduction in the juice content (%) with increasing 2,4-D concentrations at both timings was observed (P = 0.0001). Granulation was not visible in any of the fruit sampled.

'Newhall' navel orange

2007/2008: The application of 2,4-D had no effect on the fruit diameter at harvest (2008/06/16) (Table 10). All the treatments except 25 mg·L^{-1} 2,4-D at 4 WAPD reduced the average navel-end size (all fruit) compared to the control. A linear increase in the average navel-end size (all fruit) over the time of 2,4-D application was observed (P = 0.0048). There was no significant difference in average navel-end size of only fruit with open navel-ends between the control and the treated fruit. Only 25 mg·L^{-1} 2,4-D at FB and 25 mg·L^{-1} 2,4-D at PD with 10 mg·L^{-1} GA significantly increased the percentage of closed navel-ends (P = 0.0001). The percentage of closed navel-ends decreased linearly over the time of 2,4-D application (P = 0.0001). No significant treatment effects were observed on the yield per tree and the total fruit number per tree.

There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 11). Although there were significant treatment effects, there was no clear trend in the fruit shape due to 2,4-D application (Table 12). The application of 2,4-D had no effect on the central axis diameter. All the treatments reduced rind thickness but not always significantly. A linear reduction in the rind thickness over the time of 2,4-D application was observed (P = 0.0004). All the treatments except 25 mg·L^{-1} 2,4-D at FB reduced the pedicel diameter significantly. The application of 2,4-D had no effect on the ^{o}Brix, the TA and the ^{o}Brix:TA ratio (Table 13). All the treatments the treatments except 25 mg·L^{-1} 2,4-D with 10 mg·L^{-1} GA at PD and 25 mg·L^{-1} 2,4-D at 2 WAPD significantly reduced the juice percentage. Granulation was not visible in any of the fruit sampled. There was no difference in the initial fruit set between the treated and the control trees (Table 14).

2008/2009: Fruit diameter at harvest (2009/06/09) was not affected by the application of 2,4-D (Table 15). Only 25 mg·L^{-1} 2,4-D at FB or PD significantly reduced the average navel-end size for all fruit and for fruit with only open navel-ends. A linear increase in the average navel-end size (all fruit) and the average navel-end size of only fruit with open navel-ends over the time of 2,4-D application was observed. The application of 2,4-D at FB significantly

increased the percentage of closed navel-ends (P = 0.0001) with PD, 2 WAPD and 4 WAPD applications having no effect. The percentage of closed navel-ends decreased linearly over the time of 2,4-D application (P = 0.0001). No significant treatment effects were observed on the yield per tree and the total fruit number per tree.

There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 16). Only 25 mg·L^{-1} 2,4-D at FB affected fruit shape (Table 17). All the treatments except 25 mg·L^{-1} 2,4-D at 4 WAPD significantly increased the central axis diameter (P = 0.0020). The application of 2,4-D had no effect on the rind thickness and only 25 mg·L^{-1} 2,4-D at 4 WAPD reduced the pedicel diameter significantly. The application of 2,4-D had no effect on the ^{o}Brix and the TA (Table 18). Although all the treatments reduced the ^{o}Brix:TA ratio compared to the control, it was not significant. All the treatments except 25 mg·L^{-1} 2,4-D at 4 WAPD significantly reduced the juice content (%) compared to the control. Granulation was not visible in any of the fruit sampled.

'Navelina' navel orange

2007/2008: The application of different concentrations of 2,4-D at PD had no effect on the fruit diameter at harvest (2008/06/13), the average navel-end size (all fruit), the average navel-end size of only fruit with open navel-ends, the percentage of closed navel-ends, the yield per tree and the total fruit number per tree (Table 19). The application of 2,4-D had no effect on the fruit rind colour and the colour at the navel-end (Table 20). Only 15 mg·L^{-1} 2,4-D increased both creasing severity and creasing incidence whilst 45 mg·L^{-1} significantly decreased both parameters. A linear reduction in both the creasing severity and the creasing incidence was observed due to increasing 2,4-D concentration (P = 0.0001). The application of 2,4-D at PD had no effect on the fruit shape (Table 21). Only 45 mg·L^{-1} 2,4-D at PD significantly increased the central axis diameter compared to the control. All the treatments except 15 mg·L^{-1} and 45 mg·L^{-1} 2,4-D at PD reduced the rind thickness compared to the control. The application of 25 mg·L^{-1} 2,4-D with 10 mg·L^{-1} GA and 45 mg·L^{-1} 2,4-D at PD significantly increased the pedicel diameter compared to the control.

There were no significant differences in the ^{o}Brix, the TA and the ^{o}Brix:TA ratio between the treated and the control fruit (Table 22). Although 25 mg·L^{-1} 2,4-D with 10 mg·L^{-1} GA and 35 mg·L^{-1} 2,4-D significantly reduced the juice content (%) compared to the control, granulation

was not visible in any of the fruit sampled. The addition of GA to 2,4-D significantly increased initial fruit set (P = 0.0002) (Table 23), but had no effect on the yield per tree and the final fruit number per tree (Table 19).

2008/2009: The application of 2,4-D had no effect on the fruit diameter at harvest (2009/06/08), the average navel-end size (all fruit), the average navel-end size of fruit with only open navel-ends, the percentage of closed navel-ends, the yield per tree and the total fruit number per tree (Table 24). A linear reduction in the average navel-end size (all fruit) was observed with increasing 2,4-D concentration and the percentage of closed navel-ends increased linearly with increasing 2,4-D concentration. There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 25). The application of 2,4-D had no effect on the fruit shape and the central axis diameter (Table 26). Only 35 $mg \cdot L^{-1}$ 2,4-D significantly reduced the rind thickness compared to the control. Two treatments, 25 $mg \cdot L^{-1}$ and 45 $mg \cdot L^{-1}$ 2,4-D significantly reduced the pedicel diameter compared to the control.

There were no differences in the ^{o}Brix and the juice content (%) between treated and control fruit (Table 27). The TA was significantly reduced by the application of 25 $mg \cdot L^{-1}$ and 45 $mg \cdot L^{-1}$ 2,4-D at PD. A linear reduction in the TA was observed with increasing 2,4-D concentration. Only 45 $mg \cdot L^{-1}$ 2,4-D significantly increased the ^{o}Brix:TA ratio. The ^{o}Brix:TA ratio increased linearly with increasing 2,4-D concentration. Granulation was not visible in any of the fruit sampled.

'Palmer' navel orange

2007/2008: The application of 2,4-D had no effect on the fruit diameter at harvest (2008/06/19), the average navel-end size (all fruit), the average navel-end size of only fruit with open navel-ends and the percentage of closed navel-ends (Table 28). There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 29). In addition, 2,4-D had no effect on the fruit shape and on the pedicel diameter (Table 30). The central axis diameter and the rind thickness were significantly greater in the treated fruit compared to the control (P = 0.0001). There were no significant differences in the ^{o}Brix, the TA and the ^{o}Brix:TA ratio between the treated and the control fruit (Table 31) and although there were

significant treatment effects, there was no clear trend in juice content (%) due to treatment with 2,4-D. Granulation was not visible in any of the sampled fruit.

2008/2009: The fruit diameter at harvest (2009/06/09), the average navel-end size (all fruit) and the average navel-end size of only fruit with open navel-ends were not affected by treatment with 2,4-D (Table 32). Although all the 2,4-D treatments increased the percentage of closed navel-ends, only 25 $mg \cdot L^{-1}$ and 30 $mg \cdot L^{-1}$ at PD were significant. There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 33). The application of 2,4-D had no effect on the fruit shape, the central axis diameter, the rind thickness and the pedicel diameter (Table 34). Similarly, there were no significant differences in the ^{o}Brix, the TA, the ^{o}Brix:TA ratio and the juice content (%) between the treated and the control fruit (Table 35). Granulation was not visible in any of the sampled fruit.

'Autumn Gold' navel orange

2008/2009: The application of 2,4-D significantly increased fruit diameter at harvest (2009/07/16) in all treatments except for 25 $mg \cdot L^{-1}$ at PD (Table 36). Both FB and PD applications reduced the average navel-end size (all fruit) and increased the percentage of closed navel-ends in all the treatments compared to the control. The two formulations of 2,4-D were equally effective in reducing the average navel-end size (all fruit) and increasing the percentage of closed navel-ends. The FB applications were more effective than the PD application in increasing the percentage of closed navel-ends ($P = 0.0015$). There was no difference in average navel-end size for only the fruit with open navel-ends between the treated and the control fruit.

There were no significant differences in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between the treated and the control fruit (Table 37). The application of 2,4-D had no effect on the fruit shape, the rind thickness and the pedicel diameter (Table 38). The central axis diameter was significantly larger in all treated fruit compared to the control fruit ($P = 0.0002$). There were no significant differences in the ^{o}Brix, the TA and the ^{o}Brix:TA ratio between the treated and the control fruit (Table 39). Juice content (%) was significantly lower in all the treated fruit compared to the control ($P = 0.0001$). Granulation was not visible in any of the sampled fruit.

'Robyn' navel orange

2007/2008: Although there were significant treatment effects on the fruit diameter at harvest (2008/07/29), there was no clear trend between the treated and the control fruit (Table 40). The application of 2,4-D at FB significantly reduced the average navel-end size (all fruit) with the PD and the 2 WAPD applications having no effect and the 4 WAPD application increasing the average navel-end size compared to the control. A linear increase in the average navel-end size (all fruit) over time the time of 2,4-D application was observed (P = 0.0001). There was no difference in the average navel-end size of only the fruit with open navel-ends between treated and control fruit. The percentage of closed navel-ends was significantly increased by both applications of 2,4-D at FB (P = 0.0030). The percentage of closed navel-ends decreased linearly over the time of 2,4-D application (P = 0.0002). No significant treatment effects were observed on the yield per tree and the total fruit number per tree.

There was no difference in the fruit rind colour, the colour at the navel-end, creasing severity and creasing incidence between treated and control fruit (Table 41). Fruit shape was not affected by the application of 2,4-D (Table 42). Although some treatments significantly differed from the control, there was no apparent trend due to 2,4-D application on the central axis diameter or the pedicel diameter. Although all the treatments reduced the rind thickness, only the PD and the 4 WAPD treatments were significant.

The ^{o}Brix and the TA were not affected by the application of 2,4-D (Table 43). Although both PD applications increased the ^{o}Brix:TA ratio, there was no clear trend. The juice content (%) was significantly reduced by 25 mg·L^{-1} at PD, 2 WAPD or 4 WAPD and a quadratic decrease in the juice content over time was observed due to 2,4-D application. The higher concentration at PD (25 mg·L^{-1}) significantly reduced the juice percentage compared to the lower concentration (20 mg·L^{-1}). However, granulation was not visible in any of the fruit sampled. There was no difference in the initial fruit set between treated and control trees for the FB treatments (Table 44).

Postharvest fruit storage quality and peelabilty of the fruit

In all the cultivars, at both evaluation dates, the treated and control fruit showed no signs of chilling injury, stem-end rind breakdown and postharvest pitting and there was no difference

in calyx condition between the treated and the control fruit (2007/2008 season) (data not shown). Similar results were obtained for 'Washington' and 'Palmer' navel orange in the 2008/2009 season. Peelabilty of the fruit was not affected by 2,4-D application (data not shown).

Gas exchange measurements and residue analysis

The application of 2,4-D had no effect on the $A_{sat,}$, A_{max} and R_d using the gas exchange method (Table 45). The residue analysis detected minute levels of 2,4-D (<0.01 mg/kg) in the treated fruit.

General results

In addition, the following general observations were noted in both seasons and in all the cultivars. The application of 2,4-D damaged the young leaves on the new growth flushes, but had no effect on the mature leaves. The damage was caused by the epinastic reaction of leaves to 2,4-D which resulted in a downward and inward rolling of the young leaves at the margins and was more pronounced at higher concentrations (Fig. 1). When sprayed at FB, 2,4-D delayed the petal drop (Fig. 2). The application of 2,4-D caused the styles to persist on the fruit, in some cases up to fruit maturity especially on 'Autumn Gold' navel orange (Fig. 3). Rind coarseness was not visibly affected by the application of 2,4-D in all the cultivars.

Discussion

The application of 2,4-D had no effect on the fruit diameter except for 'Autumn Gold' navel orange where it increased the fruit diameter. Previous studies reported that 2,4-D increased fruit size (Anthony and Coggins, 1999; Guardiola, 1997; Stewart and Klotz, 1947). However, Guardiola (1997) reported that not all cultivars respond to the growth enhancing effect of 2,4-D. The application of 2,4-D at FB consistently reduced the average navel-end size (all fruit) in all the cultivars and in both seasons. This concurs with previous results obtained by Gardiazabal (2006) and Saveedra (2006). The application of 2,4-D at PD did not reduce the average navel-end size (all fruit) except for 'Autumn Gold', 'Newhall' and 'Washington' (2008/2009 season) navel, although previous studies indicated that 2,4-D is effective at PD (Verreynne, 2008). The late applications of 2,4-D at 2 WAPD and 4 WAPD had no effect on the average navel-end size (all fruit). The effectivity of 2,4-D decreased over time after

anthesis with the best results obtained at FB, with later applications being generally ineffective.

The average navel-end size of the open navel-ends was not reduced by 2,4-D except for 'Washington' (2008/2009 season) and 'Newhall' (2008/2009 season) navel oranges. Therefore, it seems the 2,4-D completely closes the navel-end rather than making it smaller as it has no effect on the average size of the open navel-ends. The application of 2,4-D at FB consistently increased the percentage of closed navel-ends in both seasons with PD applications being effective on 'Palmer' (2008/2009 season), 'Autumn Gold' and 'Newhall' (2008/2009 season) navel orange. Gardiazabal (2006) and Saavedra (2006) reported that FB applications of 2,4-D increased the percentage of closed navel-ends, with Verreynne (2008) reporting the PD applications also being effective. The application of 2,4-D did not affect the total fruit number per tree or the yield per tree. Similar results were reported by Gardiazabal (2006). The application of 2,4-D after physiological fruit drop resulted in a fruit thinning effect (Stewart et al., 1951), but this was not observed in our study as we had earlier applications.

The application of 2,4-D had no effect on the fruit rind colour, however greener coloured fruit has been reported after 2,4-D application (Stewart et al., 1951; Verreynne, 2008). The colour at the navel-end was not affected by 2,4-D. Verreynne (2008) reported greener navel-ends in fruit treated with 2,4-D. The application of 2,4-D had no effect on the creasing severity and the creasing incidence expect for a reduction seen in 'Washington' (2007/2008 season) and 'Navelina' navel orange (2007/2008 season). Similarly, the application of 2,4-D did not have any effect on creasing in 'Lanelate' navel orange (Saavedra 2006) and 'Nova' mandarin (Greenberg et al., 2006).

The application of 2,4-D had no effect on the fruit shape, with similar results have been reported by Verreynne (2008), whilst Stewart et al. (1951) reported the application of 2,4-D at high concentrations (75 $mg \cdot L^{-1}$ or 225 $mg \cdot L^{-1}$) caused the fruit to become cylindrical in shape. The application of 2,4-D increased the central axis diameter in 'Palmer' (2007/2008 season), Autumn Gold (2008/2009 season), 'Washington' (2008/2009 season) and 'Newhall' (2008/2009 season) navel orange. This increase in central axis diameter may be of concern as it may reduce the juice content of the fruit. Rind thickness was reduced in 'Robyn' and 'Washington' (2008/2009 season) navel orange, but was increased in 'Palmer' navel (2008/2009 season). Previous studies reported an increased rind thickness due to 2,4-D

application (Stewart et al., 1951), although at much higher concentration (> 75 mg·L^{-1}). The pedicel diameter was not affected by 2,4-D application, except for 'Newhall' navel orange (2007/2008 season) in which treated fruit had thinner pedicels than the control fruit. This contradicts previous studies by Stewart et al. (1951) who reported thicker pedicels in 2,4-D treated fruit.

In terms of internal fruit quality, the application of 2,4-D did not affect the ^{o}Brix and the TA concurring with Gardiazabal (2006) and Verreynne (2008). The ^{o}Brix:TA ratio was not affected in all trials except for 'Navelina' (2008/2009 season) and 'Robyn' (2007/2008 season) navel orange where the treatment with 2,4-D increased the ^{o}Brix:TA ratio. The early application of 2,4-D at FB reduced the juice content (%) of the fruit only in 'Autumn Gold' and 'Newhall' navel orange, with later applications generally reducing juice content (%) in all the cultivars, although not always significant. A reduction in juice content (%) by 2,4-D was previously reported (Stewart and Klotz, 1947; Stewart et al., 1951; Verreynne 2008). Although the application of 2,4-D was reported to reduce granulation on Valencia oranges (Coggins and Hield, 1968), granulation was not visible in both treated and control fruit in all the trials. The postharvest storage quality, as seen by the lack of chilling injury or rind disorders and the peelabilty of the fruit were not affected by the prehaverst application of 2,4-D in all the cultivars.

The application of 2,4-D at FB increased the initial fruit set in 'Washington' navel orange, whilst the addition of GA to 2,4-D at PD also increased the fruit set in 'Navelina' navel orange. Previous studies reported that GA increases fruit set in citrus (Davies, 1986; Moss, 1972). However, yield and the total number of fruit per tree at harvest were not affected despite the increase in the initial fruit set due to 2,4-D. The increase in fruit set due to 2,4-D is temporary (Stewart and Klotz, 1947), with 2,4-D delaying the normal fruitlet abscission.

Although 2,4-D damaged the young leaves on new growth flushes that were present at the time of spraying, the damage was not visible at the time of harvest, suggesting it was temporary as no leaf abscission occurred. The leaf damage was more pronounced at higher concentrations of 2,4-D and barely visible at low concentrations. Leaf damage did not affect tree productivity as no yield losses were observed. Citrus trees produce several new growth flushes per year minimising the effect of leaf damage on tree productivity (Stewart et al., 1951). In addition, gas exchange measurements reported no differences in photosynthetic rates between the treated and control leaves. Previous studies reported that 2,4-D inhibited the

photosynthesis of detached 'Washington' navel orange leaves (Wedding et al., 1954). No detectable 2,4-D residues were found in fruit at harvest, after the prehaverst application of 2,4-D at 45 mg·L^{-1} at PD on 'Navelina' navel orange.

The persistence of styles on the navel orange fruit treated with 2,4-D was reported previously by Krezdorn (1969) and Verreynne (2008). The mode of action of 2,4-D in closing the navel-end appears to be related to the delayed abscission of the style due to 2,4-D treatment (Verreynne 2008). If the style abscises early during fruit development more open navel-ends are likely to be formed than if the style stays attached for a longer time as the style helps to keep the navel-end intact. The delayed abscission of the style may be linked to the delay in the formation of the abscission layer between the fruit and the style due to 2,4-D treatment. Normally, cellulase breaks down the vascular bundles connecting the style to the fruit leading to the formation of the abscission layer, but the application of 2,4-D may decrease fruit ethylene levels which in turn suppresses cellulase activity (Lima and Davies 1984b). Rind coarseness was not visibly affected in any of the treated fruit, although 2,4-D had been reported to increase rind coarseness on fruit at much higher concentrations (> 75 mg·L^{-1}) (Stewart and Klotz, 1947).

In conclusion, the application of 2,4-D at FB consistently decreased the average navel-end size (all fruit) and increased the percentage of closed navel-ends in all the cultivars and in all the different production areas, with later applications being generally ineffective. It seems that once style abscission commenced, the 2,4-D treatments became ineffective in reducing the percentage of closed navel-ends. Therefore, the timing of the application was more important than the concentration applied, with no differences between the different concentrations at each timing. There were no major negative side effects on internal and external fruit quality except for the reduction in juice percentage (%), especially with the later treatments at PD, 2 WAPD and 4 WAPD. Therefore the application of 15 mg·L^{-1} 2,4-D at FB can be used to increase the percentage of closed navel-ends and possibly reduce fruit splitting in citrus, mitigate stylar-end decay, improve insect control and improve the export packout by reducing the amount of fruit culled in the packhouse. Future work should include the use of lower concentrations of 2,4-D at FB and treatments with the amine formulation to address the concern growers might have around the volatility of the ester formulation especially in cases where there are susceptible plants near the orchard like gravepines.

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Table 1. Effect of 2,4-D on the fruit diameter, navel-end size and the percentage of closed navel-ends of 'Washington' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navel-ends)	Closed navels
	----------*mm*----------			--%--
Control	71.25	3.71 a[z]	5.11	28.36 bc
15 mg·L^{-1} 2,4-D at FB	70.69	2.07 bc	4.00	53.33 a
25 mg·L^{-1} 2,4-D at FB	73.28	2.05 bc	4.47	49.87 a
35 mg·L^{-1} 2,4-D at FB	72.16	1.79 c	3.92	54.23 a
15 mg·L^{-1} 2,4-D at PD	70.43	3.24 ab	4.44	26.72 bc
25 mg·L^{-1} 2,4-D at PD	70.87	3.08 ab	5.20	39.69 ab
35 mg·L^{-1} 2,4-D at PD	71.38	3.67 a	4.63	19.76 c
P- value	0.3799	0.0071	0.6070	0.0005
Contrast				
Control vs. 2,4-D	0.8342	0.0270	0.2784	0.0647
FB vs. PD	0.1439	0.0004	0.1876	0.0001
2,4-D linear	0.2055	0.8641	0.9283	0.6240
2,4-D quadratic	0.2717	0.7354	0.2406	0.2349
Timing * 2,4-D linear	0.7864	0.4083	0.8126	0.5018
Timing * 2,4-D quadratic	0.2526	0.5086	0.8830	0.0555

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 2. Effect of 2,4-D on the external fruit quality of 'Washington' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	1.55	0.88	0.78	47.22
15 mg·L^{-1} 2,4-D at FB	2.05	1.01	0.90	52.78
25 mg·L^{-1} 2,4-D at FB	1.86	0.83	0.67	38.89
35 mg·L^{-1} 2,4-D at FB	1.73	0.95	0.76	44.44
15 mg·L^{-1} 2,4-D at PD	1.45	0.47	1.03	58.33
25 mg·L^{-1} 2,4-D at PD	1.61	1.01	1.25	70.83
35 mg·L^{-1} 2,4-D at PD	1.83	0.75	0.68	38.89
P- value	0.1676	0.1851	0.2081	0.0885
Contrast				
Control vs. 2,4-D	0.2465	0.7691	0.5784	0.6917
FB vs. PD	0.0645	0.1365	0.1476	0.1148
2,4-D linear	0.8629	0.4702	0.1671	0.0940
2,4-D quadratic	0.8033	0.3497	0.4468	0.3795
Timing * 2,4-D linear	0.0375	0.2814	0.5485	0.4943
Timing * 2,4-D quadratic	1.0000	0.0432	0.0682	0.0288

Means were separated at the 5% level (LSD)
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
FB (full bloom)
PD (100% petal drop)

Table 3. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Washington' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.98	13.16 abc[z]	5.61 b	2.94
15 mg·L^{-1} 2,4-D at FB	1.00	12.34 bc	5.64 b	2.78
25 mg·L^{-1} 2,4-D at FB	1.01	15.23 a	6.65 a	3.09
35 mg·L^{-1} 2,4-D at FB	0.99	10.74 c	5.25 b	2.74
15 mg·L^{-1} 2,4-D at PD	1.00	10.94 c	4.98 b	2.71
25 mg·L^{-1} 2,4-D at PD	0.99	12.25 bc	5.21 b	3.18
35 mg·L^{-1} 2,4-D at PD	0.99	13.99 ab	5.63 b	2.93
P- value	0.5753	0.0111	0.0319	0.1899
Contrast				
Control vs. 2,4-D	0.1933	0.5485	0.8415	0.7775
FB vs. PD	0.3687	0.5928	0.0729	0.5740
2,4-D linear	0.3422	0.4133	0.8401	0.5543
2,4-D quadratic	0.8376	0.0282	0.0887	0.0091
Timing * 2,4-D linear	0.9557	0.0126	0.1661	0.3560
Timing * 2,4-D quadratic	0.2748	0.0147	0.0190	0.8927

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
FB (full bloom)
PD (100% petal drop)

Table 4. Effect of 2,4-D on the internal fruit quality of 'Washington' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	12.06	0.87	13.93	42.63
15 mg·L^{-1} 2,4-D at FB	11.38	0.81	13.92	41.93
25 mg·L^{-1} 2,4-D at FB	11.38	0.92	12.53	42.19
35 mg·L^{-1} 2,4-D at FB	12.08	0.84	14.36	41.49
15 mg·L^{-1} 2,4-D at PD	11.88	0.84	14.14	42.21
25 mg·L^{-1} 2,4-D at PD	12.30	0.84	14.65	39.04
35 mg·L^{-1} 2,4-D at PD	11.78	0.82	14.37	42.06
P- value	0.1214	0.5763	0.1886	0.3908
Contrast				
Control vs. 2,4-D	0.3520	0.5198	0.9157	0.3634
FB vs. PD	0.0879	0.4338	0.0915	0.4225
2,4-D linear	0.2549	0.8890	0.5399	0.7986
2,4-D quadratic	0.7962	0.1572	0.2127	0.1990
Timing * 2,4-D linear	0.1322	0.5913	0.8556	0.9014
Timing * 2,4-D quadratic	0.0781	0.2565	0.0448	0.0828

Means were separated at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 5. Effect of 2,4-D on the fruit set of 'Washington' navel orange in the Citrusdal area, South Africa (2007-2008).

Treatment	Fruit set
	--%--
Control	43.66 b[z]
15 mg·L^{-1} 2,4-D at FB	63.46 a
25 mg·L^{-1} 2,4-D at FB	62.13 a
35 mg·L^{-1} 2,4-D at FB	70.75 a
P- value	0.0005
Contrast	
Control vs. 2,4-D	0.0001
2,4-D linear	0.2629
2,4-D quadratic	0.3775

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 6. Effect of 2,4-D on the fruit diameter, navel size and the percentage closed navels of 'Washington' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navels)	Closed navels
	----------*mm*----------			*--%--*
Control	77.24	8.91 a[z]	9.75 a	8.73 b
15 mg·L^{-1} 2,4-D at FB	78.86	4.99 cd	7.66 bc	36.03 a
25 mg·L^{-1} 2,4-D at FB	79.82	4.08 d	6.43 c	33.82 a
35 mg·L^{-1} 2,4-D at FB	78.94	3.82 d	7.64 bc	46.16 a
15 mg·L^{-1} 2,4-D at PD	82.19	7.99 ab	8.78 ab	8.70 b
25 mg·L^{-1} 2,4-D at PD	79.33	6.90 b	8.22 abc	15.93 b
35 mg·L^{-1} 2,4-D at PD	76.07	6.44 bc	7.65 bc	15.92 b
P- value	0.3876	0.0001	0.0424	0.0013
Contrast				
Control vs. 2,4-D	0.1641	0.0001	0.0079	0.0088
FB vs. PD	0.5936	0.0001	0.0859	0.0001
2,4-D linear	0.1852	0.0169	0.3996	0.1457
2,4-D quadratic	0.9626	0.5059	0.2976	0.7203
Timing * 2,4-D linear	0.1619	0.7346	0.4049	0.8044
Timing * 2,4-D quadratic	0.5153	0.9902	0.2989	0.2885

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 7. Effect of 2,4-D on external fruit quality of 'Washington' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit rind colour[y]	Colour at navel-end[x]	Creasing severity[w]	Creasing incidence
				--%--
Control	1.77	1.20	0.94 a[z]	56.25 a
15 mg·L^{-1} 2,4-D at FB	1.99	1.16	0.86 ab	45.83 ab
25 mg·L^{-1} 2,4-D at FB	2.32	1.20	0.88 a	50.00 ab
35 mg·L^{-1} 2,4-D at FB	2.10	1.28	0.54 bc	41.66 ab
15 mg·L^{-1} 2,4-D at PD	1.91	1.14	0.45 cd	37.50 b
25 mg·L^{-1} 2,4-D at PD	2.08	1.35	0.51 cd	44.79 ab
35 mg·L^{-1} 2,4-D at PD	1.94	1.34	0.18 d	6.25 c
P- value	0.1543	0.6035	0.0002	0.0001
Contrast				
Control vs. 2,4-D	0.0585	0.6706	0.0065	0.0039
FB vs. PD	0.1502	0.4297	0.0003	0.0010
2,4-D linear	0.5957	0.1075	0.0161	0.0030
2,4-D quadratic	0.0711	0.5960	0.0707	0.0046
Timing * 2,4-D linear	0.7615	0.6830	0.8296	0.0206
Timing * 2,4-D quadratic	0.6302	0.4447	0.9336	0.0948

[z] Means with a different letter differ significantly at the 5% level (LSD)
[y] 1-8 on CRI colour chart no. 34, 1-orange, 8-green
[x] 0-4: 0-orange, 4-green
[w] 0-4: 0-no creasing, 4-whole fruit creased
FB (full bloom)
PD (100% petal drop)

Table 8. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Washington' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.99	12.71 b[z]	6.03 b	2.79 b
15 mg·L^{-1} 2,4-D at FB	1.01	12.77 b	4.85 d	2.54 b
25 mg·L^{-1} 2,4-D at FB	1.00	17.86 a	6.22 a	2.50 b
35 mg·L^{-1} 2,4-D at FB	0.98	16.01 a	5.64 bc	2.70 b
15 mg·L^{-1} 2,4-D at PD	1.01	17.00 a	4.98 d	2.56 b
25 mg·L^{-1} 2,4-D at PD	0.99	17.97 a	4.91 d	2.69 b
35 mg·L^{-1} 2,4-D at PD	0.98	16.04 a	5.27 cd	3.47 a
P- value	0.0682	0.0002	0.0001	0.0001
Contrast				
Control vs. 2,4-D	0.6226	0.0010	0.0004	0.6708
FB vs. PD	0.8199	0.0622	0.0007	0.0007
2,4-D linear	0.0021	0.2286	0.0031	0.0001
2,4-D quadratic	0.3230	0.0040	0.0132	0.0275
Timing * 2,4-D linear	0.7178	0.0290	0.1632	0.0014
Timing * 2,4-D quadratic	0.4000	0.2177	0.0003	0.2783

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
FB (full bloom)
PD (100% petal drop)

Table 9. Effect of 2,4-D on the internal fruit quality of 'Washington' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	11.67	1.00	11.69	41.43 c[z]
15 mg·L^{-1} 2,4-D at FB	11.43	1.00	11.60	44.75 ab
25 mg·L^{-1} 2,4-D at FB	10.76	0.97	11.34	42.37 bc
35 mg·L^{-1} 2,4-D at FB	11.59	0.96	12.11	42.03 bc
15 mg·L^{-1} 2,4-D at PD	11.30	1.01	11.30	45.52 a
25 mg·L^{-1} 2,4-D at PD	11.07	1.00	11.04	39.35 cd
35 mg·L^{-1} 2,4-D at PD	11.85	1.06	11.29	36.48 d
P- value	0.0819	0.5680	0.6259	0.0001
Contrast				
Control vs. 2,4-D	0.2360	0.9427	0.5912	0.7800
FB vs. PD	0.5152	0.0994	0.1662	0.0049
2,4-D linear	0.1862	0.8070	0.5427	0.0001
2,4-D quadratic	0.0082	0.5148	0.2839	0.1560
Timing * 2,4-D linear	0.4537	0.2344	0.5236	0.0051
Timing * 2,4-D quadratic	0.6027	0.7955	0.7000	0.7356

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 10. Effect of 2,4-D on the fruit diameter, navel-end size, the percentage of closed navel-ends, yield and the total fruit number of 'Newhall' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navel-ends)	Closed navel-ends	Yield	Total fruit number
	----------*mm*----------			*--%--*	*--kg.tree^{-1}--*	
Control	74.93	7.31 a[z]	8.39	12.93 c	135.91	595
25 mg·L^{-1} 2,4-D at FB	75.57	4.73 c	7.42	36.24 a	124.07	561
25 mg·L^{-1} 2,4-D at PD	75.72	5.41 bc	6.76	20.02 bc	124.16	554
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	75.74	5.35 c	6.92	24.41 b	110.12	464
25 mg·L^{-1} 2,4-D at 2 WAPD	74.09	5.82 bc	7.11	17.63 bc	119.83	533
25 mg·L^{-1} 2,4-D at 4 WAPD	74.20	6.76 ab	7.88	14.42 c	127.93	574
P-value	0.8293	0.0057	0.2156	0.0001	0.2452	0.3395
Contrast						
Control vs. 2,4-D	0.9159	0.0028	0.0418	0.0016	0.0715	0.2216
Timing linear	0.2766	0.0048	0.4483	0.0001	0.8234	0.9136
Timing quadratic	0.9865	0.7905	0.1712	0.0159	0.5821	0.5767

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 11. Effect of 2,4-D on the external quality of 'Newhall' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	1.74	0.68	0.21	14.58
25 mg·L^{-1} 2,4-D at FB	1.56	0.91	0.23	17.29
25 mg·L^{-1} 2,4-D at PD	1.27	0.74	0.21	19.22
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	1.91	0.71	0.21	15.63
25 mg·L^{-1} 2,4-D at 2 WAPD	1.61	0.71	0.37	28.12
25 mg·L^{-1} 2,4-D at 4 WAPD	1.49	0.68	0.22	16.67
P- value	0.0627	0.2228	0.8178	0.6661
Contrast				
Control vs. 2,4-D	0.2807	0.3569	0.7129	0.4794
Timing linear	0.8671	0.0350	0.7517	0.7993
Timing quadratic	0.5728	0.3160	0.4708	0.2824

Means were separated at the 5% level (LSD)
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 12. Effect of 2,4-D on fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Newhall' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.91 ab[z]	14.41	7.27 a	3.36 a
25 mg·L^{-1} 2,4-D at FB	0.88 c	14.37	7.19 a	3.20 ab
25 mg·L^{-1} 2,4-D at PD	0.89 bc	13.75	6.81 a	2.93 c
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	0.90 abc	13.19	6.10 b	2.89 c
25 mg·L^{-1} 2,4-D at 2 WAPD	0.92 a	15.84	7.09 a	2.66 d
25 mg·L^{-1} 2,4-D at 4 WAPD	0.91 ab	14.20	5.96 b	3.02 bc
P- value	0.0034	0.1509	0.0001	0.0001
Contrast				
Control vs. 2,4-D	0.1391	0.8543	0.0050	0.0001
Timing linear	0.0004	0.6069	0.0004	0.0089
Timing quadratic	0.1592	0.4524	0.0630	0.0001

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 13. Effect of 2,4-D on the internal fruit quality of 'Newhall' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	9.89	0.81	12.16	45.07 a[z]
25 mg·L^{-1} 2,4-D at FB	9.98	0.83	12.03	42.58 c
25 mg·L^{-1} 2,4-D at PD	10.03	0.84	11.96	42.27 c
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	10.00	0.85	11.88	44.74 ab
25 mg·L^{-1} 2,4-D at 2 WAPD	9.75	0.82	11.88	45.28 a
25 mg·L^{-1} 2,4-D at 4 WAPD	9.83	0.85	11.53	42.89 bc
P- value	0.9544	0.8789	0.8407	0.0065
Contrast				
Control vs. 2,4-D	0.9062	0.4142	0.4157	0.0533
Timing linear	0.5095	0.6257	0.2903	0.2113
Timing quadratic	0.9357	0.6339	0.6837	0.1412

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (Full bloom)
PD (100% petal drop)
2WAPD (2 weeks after 100% petal drop)
4WAPD (4 weeks after 100% petal drop)

Table 14. Effect of 2,4-D on the fruit set of ‘Newhall’ navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit set
	--%--
Control	67.48
25 mg·L^{-1} 2,4-D at FB	66.83
P- value	0.9267

Means were separated at the 5% level (LSD)
FB (Full bloom)

Table 15. Effect of 2,4-D on the fruit diameter, navel-end size, the percentage of closed navel-ends, yield and the total fruit number of 'Newhall' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navel-ends)	Closed navel-ends	Yield	Total number of fruit
	----------*mm*----------			*--%--*	*--kg.tree^{-1}--*	
Control	81.88	8.50 a[z]	9.56 a	10.90 b	105.93	376
25 mg·L^{-1} 2,4-D at FB	81.36	3.91 c	6.94 c	41.11 a	119.03	417
25 mg·L^{-1} 2,4-D at PD	82.60	6.02 c	7.26 bc	18.67 b	96.81	345
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	83.04	7.12 ab	8.92 ab	13.70 b	104.15	337
25 mg·L^{-1} 2,4-D at 2 WAPD	83.43	7.18 ab	7.95 abc	10.00 b	93.97	336
25 mg·L^{-1} 2,4-D at 4 WAPD	79.78	8.31 a	9.43 a	12.28 b	124.12	473
P-value	0.6379	0.0005	0.0389	0.0001	0.4369	0.4346
Contrast						
Control vs. 2,4-D	0.8845	0.0172	0.0734	0.0555	0.7879	0.8173
Timing linear	0.4131	0.0001	0.0218	0.0001	0.8123	0.4693
Timing quadratic	0.1964	0.4464	0.4171	0.0016	0.0828	0.1121

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 16. Effect of 2,4-D on the external quality of 'Newhall' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	1.15	0.18	0.17	13.10
25 mg·L^{-1} 2,4-D at FB	1.31	0.48	0.22	15.69
25 mg·L^{-1} 2,4-D at PD	1.38	0.46	0.19	13.54
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	1.45	0.51	0.02	1.43
25 mg·L^{-1} 2,4-D at 2 WAPD	1.32	0.68	0.01	1.04
25 mg·L^{-1} 2,4-D at 4 WAPD	1.25	0.75	0.11	8.33
P- value	0.1965	0.1232	0.4325	0.2272
Contrast				
Control vs. 2,4-D	0.0415	0.0398	0.4936	0.3276
Timing linear	0.5215	0.2839	0.1720	0.1203
Timing quadratic	0.3478	0.7063	0.4233	0.3250

Means were separated at the 5% level (LSD)
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 17. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Newhall' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.94 a[z]	14.86 c	6.49	3.30 ab
25 mg·L^{-1} 2,4-D at FB	0.91 b	16.79 b	6.88	3.28 ab
25 mg·L^{-1} 2,4-D at PD	0.92 ab	18.52 a	6.69	3.39 a
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	0.93 ab	17.00 ab	7.25	3.21 ab
25 mg·L^{-1} 2,4-D at 2 WAPD	0.93 ab	16.49 b	7.16	2.89 bc
25 mg·L^{-1} 2,4-D at 4 WAPD	0.94 a	16.06 bc	7.09	2.68 c
P- value	0.0484	0.0020	0.1216	0.0350
Contrast				
Control vs. 2,4-D	0.1270	0.0058	0.0665	0.1120
Timing linear	0.0024	0.0218	0.4223	0.0010
Timing quadratic	0.8254	0.0748	0.5844	0.2961

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 18. Effect of 2,4-D on the internal fruit quality of 'Newhall' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	9.54	0.74	13.26	42.95 a[z]
25 mg·L^{-1} 2,4-D at FB	9.37	0.79	11.84	39.08 b
25 mg·L^{-1} 2,4-D at PD	9.69	0.88	11.23	35.00 c
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	9.41	0.84	11.43	33.26 c
25 mg·L^{-1} 2,4-D at 2 WAPD	9.34	0.79	11.78	39.20 b
25 mg·L^{-1} 2,4-D at 4 WAPD	9.71	0.77	12.64	41.94 a
P- value	0.5522	0.0797	0.1391	0.0001
Contrast				
Control vs. 2,4-D	0.8692	0.0694	0.0312	0.0001
Timing linear	0.4511	0.2486	0.2070	0.0020
Timing quadratic	0.9569	0.0723	0.1665	0.0012

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 19. Effect of 2,4-D on the fruit diameter, navel-end size, the percentage of closed navel-ends, yield and the total fruit number of 'Navelina' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navel-ends)	Closed navels	Yield	Total fruit number
	----------*mm*----------			*--%--*	*--kg.tree^{-1}--*	
Control	75.05	6.37	7.70	21.14	74.94	317
15 mg·L^{-1} 2,4-D at PD	75.70	5.68	9.34	30.36	91.07	375
25 mg·L^{-1} 2,4-D at PD	76.36	6.27	9.56	30.42	101.85	392
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	76.92	7.47	12.68	32.24	85.72	317
35 mg·L^{-1} 2,4-D at PD	77.64	7.45	10.58	24.44	81.69	327
45 mg·L^{-1} 2,4-D at PD	76.39	6.65	8.48	21.12	86.97	352
P- value	0.8528	0.1847	0.0768	0.4621	0.5698	0.7451
Contrast						
Control vs. 2,4-D	0.3365	0.5937	0.0690	0.2507	0.2053	0.4495
2,4-D linear	0.6071	0.1095	0.7697	0.1507	0.4846	0.4996
2,4-D quadratic	0.5149	0.2176	0.3305	0.7440	0.7906	0.9244

Means were separated at the 5% level (LSD)
PD (100% petal drop)

Table 20. Effect of 2,4-D on the external fruit quality of 'Navelina' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit rind colour[y]	Colour at navel-end[x]	Creasing severity[w]	Creasing incidence
				--%--
Control	1.62	0.76	0.22 b[z]	19.79 b
15 mg·L^{-1} 2,4-D at PD	1.91	0.94	0.46 a	34.38 a
25 mg·L^{-1} 2,4-D at PD	2.12	0.95	0.16 bc	13.96 bc
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	1.81	0.74	0.19 bc	17.92 b
35 mg·L^{-1} 2,4-D at PD	2.00	0.81	0.14 bc	11.65 bc
45 mg·L^{-1} 2,4-D at PD	1.78	0.63	0.04 c	4.17 c
P- value	0.3770	0.1332	0.0008	0.0002
Contrast				
Control vs. 2,4-D	0.1067	0.6494	0.7372	0.4416
2,4-D linear	0.4768	0.0130	0.0001	0.0001
2,4-D quadratic	0.2148	0.2732	0.1104	0.1116

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[x]0-4: 0-orange, 4-green
[w]0-4: 0-no creasing, 4-whole fruit creased
PD (100% petal drop)

Table 21. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Navelina' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.92	14.04 bc[z]	7.46 ab	3.07 b
15 mg·L^{-1} 2,4-D at PD	0.89	15.23 ab	7.84 a	2.94 b
25 mg·L^{-1} 2,4-D at PD	0.90	13.08 c	6.06 d	3.22 b
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	0.89	13.47 c	6.82 c	3.68 a
35 mg·L^{-1} 2,4-D at PD	0.88	15.30 ab	6.52 cd	4.03 a
45 mg·L^{-1} 2,4-D at PD	0.89	16.38 a	7.07 bc	3.29 b
P- value	0.3179	0.0008	0.0001	0.0001
Contrast				
Control vs. 2,4-D	0.0280	0.2792	0.0169	0.0148
2,4-D linear	0.9690	0.0260	0.0657	0.0839
2,4-D quadratic	0.8728	0.0045	0.0001	0.3645

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
PD (100% petal drop)

Table 22. Effect of 2,4-D on the internal fruit quality of 'Navelina' navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	9.30	0.79	11.85	43.28 a[z]
15 mg·L^{-1} 2,4-D at PD	8.85	0.77	11.48	42.84 a
25 mg·L^{-1} 2,4-D at PD	9.37	0.75	12.72	42.68 a
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	9.73	0.77	12.63	39.76 b
35 mg·L^{-1} 2,4-D at PD	9.37	0.74	12.65	39.24 b
45 mg·L^{-1} 2,4-D at PD	9.06	0.71	12.70	42.12 a
P- value	0.3799	0.1308	0.2677	0.0016
Contrast				
Control vs. 2,4-D	0.9501	0.0738	0.2546	0.0270
2,4-D linear	0.6257	0.0556	0.0908	0.1153
2,4-D quadratic	0.1572	0.9391	0.2051	0.0572

[z]Means with a different letter differ significantly at the 5% level (LSD)
PD (100% petal drop)

Table 23. Effect of 2,4-D on the fruit set of ‘Navelina’ navel orange in the Citrusdal area, South Africa (2007/2008).

Treatment	Fruit set
	--%--
Control	31.77 b[z]
15 mg·L^{-1} 2,4-D at PD	40.44 b
25 mg·L^{-1} 2,4-D at PD	38.86 b
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	55.81 a
35 mg·L^{-1} 2,4-D at PD	42.01 b
45 mg·L^{-1} 2,4-D at PD	32.52 b
P- value	0.0008
Contrast	
Control vs. 2,4-D	0.0266
2,4-D linear	0.2691
2,4-D quadratic	0.3429

[z]Means with a different letter differ significantly at the 5% level (LSD)
PD (100% petal drop)

Table 24. Effect of 2,4-D on the fruit diameter, navel-end size, the percentage of closed navel-ends, yield and the total fruit number of 'Navelina' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navel-ends)	Closed navels	Yield	Total fruit number
	----------*mm*----------			*--%--*	*--kg.tree^{-1}--*	
Control	87.62	8.98	9.45	5.34	37.41	106
15 mg·L^{-1} 2,4-D at PD	90.82	11.36	11.54	1.61	37.14	98
25 mg·L^{-1} 2,4-D at PD	88.48	10.87	12.20	10.88	46.61	117
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	87.40	10.65	11.74	10.14	48.81	154
35 mg·L^{-1} 2,4-D at PD	86.64	10.75	12.14	11.31	47.26	150
45 mg·L^{-1} 2,4-D at PD	86.78	7.61	9.17	16.25	58.20	181
P- value	0.5051	0.0580	0.0735	0.0960	0.7864	0.5768
Contrast						
Control vs. 2,4-D	0.8833	0.2892	0.0866	0.2320	0.3810	0.3541
2,4-D linear	0.0775	0.0095	0.0845	0.0093	0.2272	0.0926
2,4-D quadratic	0.4581	0.1550	0.0493	0.5836	0.9172	0.8793

Means were separated at the 5% level (LSD)
PD (100% petal drop)

Table 25. Effect of 2,4-D on the external fruit quality of 'Navelina' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit rind colour[y]	Colour at navel-end[x]	Creasing severity[w]	Creasing incidence
				--%--
Control	1.25	0.33	0.02	2.38
15 mg·L^{-1} 2,4-D at PD	1.47	0.55	0.01	1.04
25 mg·L^{-1} 2,4-D at PD	1.54	0.43	0.00	0.00
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	1.50	0.42	0.00	0.00
35 mg·L^{-1} 2,4-D at PD	1.44	0.25	0.00	0.00
45 mg·L^{-1} 2,4-D at PD	1.48	0.15	0.00	0.00
P- value	0.6144	0.4293	0.1739	0.1739
Contrast				
Control vs. 2,4-D	0.1477	0.8428	0.0186	0.0186
2,4-D linear	0.9877	0.0383	0.4282	0.4282
2,4-D quadratic	0.9648	0.9389	0.4304	0.4304

Means were separated at the 5% level (LSD)
[y]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[x]0-4: 0-orange, 4-green
[w]0-4: 0-no creasing, 4-whole fruit creased
PD (100% petal drop)

Table 26. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Navelina' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.94	15.15	7.57 ab[z]	4.49 ab
15 mg·L^{-1} 2,4-D at PD	0.92	16.18	6.82 bc	4.02 bc
25 mg·L^{-1} 2,4-D at PD	0.93	17.09	8.01 a	3.94 c
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	0.95	17.29	7.82 ab	4.61 a
35 mg·L^{-1} 2,4-D at PD	0.93	15.72	6.34 c	4.48 ab
45 mg·L^{-1} 2,4-D at PD	0.94	16.44	7.81 ab	3.38 d
P- value	0.6791	0.5128	0.0298	0.0005
Contrast				
Control vs. 2,4-D	0.8658	0.1421	0.7338	0.0725
2,4-D linear	0.3902	0.5344	0.5020	0.1039
2,4-D quadratic	0.7965	0.8161	0.7494	0.0099

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
PD (100% petal drop)

Table 27. Effect of 2,4-D on the internal fruit quality of 'Navelina' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	9.51	0.72 ab[z]	13.16 bc	39.09
15 mg·L^{-1} 2,4-D at PD	9.24	0.74 a	12.50 c	38.01
25 mg·L^{-1} 2,4-D at PD	9.09	0.65 cd	14.03 ab	37.14
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	9.23	0.70 abc	13.24 abc	39.48
35 mg·L^{-1} 2,4-D at PD	9.14	0.67 bcd	13.67 ab	37.34
45 mg·L^{-1} 2,4-D at PD	8.84	0.62 d	14.35 a	37.94
P- value	0.5061	0.0040	0.0367	0.7984
Contrast				
Control vs. 2,4-D	0.0993	0.0450	0.3257	0.4282
2,4-D linear	0.1820	0.0007	0.0072	0.9405
2,4-D quadratic	0.6110	0.4374	0.3093	0.6382

[z]Means with a different letter differ significantly at the 5% level (LSD)
PD (100% petal drop)

Table 28. Effect of 2,4-D on the fruit diameter, navel-end size and the percentage of closed navel-ends of 'Palmer' navel orange in the Addo area, South Africa (2007/2008).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navels)	Closed navel-ends
	----------*mm*----------			--%--
Control	80.26	4.60	5.54	16.45
15 mg·L^{-1} 2,4-D at PD	82.99	4.04	5.57	25.52
20 mg·L^{-1} 2,4-D at PD	82.38	3.89	5.08	23.97
25 mg·L^{-1} 2,4-D at PD	81.90	3.60	5.07	29.18
30 mg·L^{-1} 2,4-D at PD	83.80	3.91	5.93	29.31
P- value	0.0760	0.4418	0.4033	0.1512
Contrast				
Control vs. 2,4-D	0.0144	0.0885	0.0145	0.0219
2,4-D linear	0.6144	0.6982	0.6714	0.3479
2,4-D quadratic	0.1586	0.5280	0.4552	0.8303

Means were separated at the 5% level (LSD)
PD (100% petal drop)

Table 29. Effect of 2,4-D on the external fruit quality of 'Palmer' navel orange in the Addo area, South Africa (2007/2008).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	2.05	0.78	0.43	25.21
15 mg·L^{-1} 2,4-D at PD	1.99	1.19	0.28	22.56
20 mg·L^{-1} 2,4-D at PD	1.91	0.89	0.26	21.53
25 mg·L^{-1} 2,4-D at PD	1.97	0.93	0.17	13.54
30 mg·L^{-1} 2,4-D at PD	2.10	0.97	0.16	14.58
P-value	0.8491	0.0705	0.2045	0.5276
Contrast				
Control vs. 2,4-D	0.6950	0.0580	0.0362	0.2549
2,4-D linear	0.4712	0.1603	0.2501	0.2799
2,4-D quadratic	0.4289	0.0857	0.9381	0.9513

Means were separated at the 5% level (LSD)
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
PD (100% petal drop)

Table 30. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Palmer' navel orange in the Addo area, South Africa (2007/2008).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	1.02	10.63 b[z]	4.04 c	3.21
15 mg·L^{-1} 2,4-D at PD	1.02	14.71 a	5.15 b	3.39
20 mg·L^{-1} 2,4-D at PD	1.04	14.51 a	4.81 b	3.34
25 mg·L^{-1} 2,4-D at PD	1.02	13.79 a	4.80 b	3.26
30 mg·L^{-1} 2,4-D at PD	1.01	14.99 a	5.52 ab	3.13
P-value	0.3748	0.0001	0.0001	0.0941
Contrast				
Control vs. 2,4-D	0.5318	0.0001	0.0001	0.3776
2,4-D linear	0.3171	0.9699	0.0712	0.0095
2,4-D quadratic	0.1840	0.2515	0.0004	0.6081

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
PD (100% petal drop)

Table 31. Effect of 2,4-D on the internal fruit quality of 'Palmer' navel orange in the Addo area, South Africa (2007/2008).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	11.10	0.90	12.59	45.60 b[z]
15 mg·L^{-1} 2,4-D at PD	11.36	0.86	13.23	46.94 ab
20 mg·L^{-1} 2,4-D at PD	11.34	0.90	12.75	40.74 c
25 mg·L^{-1} 2,4-D at PD	10.94	0.86	12.69	49.09 a
30 mg·L^{-1} 2,4-D at PD	11.16	0.82	13.61	44.41 b
P-value	0.4770	0.3909	0.4110	0.0001
Contrast				
Control vs. 2,4-D	0.6220	0.2928	0.3255	0.7749
2,4-D linear	0.2546	0.2786	0.5746	0.8547
2,4-D quadratic	0.4840	0.2060	0.1116	0.4175

[z]Means with a different letter differ significantly at the 5% level (LSD)
PD (100% petal drop)

Table 32. Effect of 2,4-D on the fruit diameter, navel-end size and the percentage closed navel-ends of 'Palmer' navel orange in the Addo area, South Africa (2008/2009).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navels)	Closed navel-ends
	----------*mm*----------			--%--
Control	77.13	4.77	6.38	24.53 b[z]
15 mg·L^{-1} 2,4-D at PD	76.30	3.51	5.93	40.12 ab
20 mg·L^{-1} 2,4-D at PD	76.44	3.92	6.57	41.58 ab
25 mg·L^{-1} 2,4-D at PD	73.75	2.40	4.63	49.32 a
30 mg·L^{-1} 2,4-D at PD	76.01	3.22	6.62	55.18 a
P- value	0.6594	0.1207	0.3003	0.0433
Contrast				
Control vs. 2,4-D	0.3468	0.0206	0.3663	0.0035
2,4-D linear	0.5826	0.3028	0.8534	0.0830
2,4-D quadratic	0.6230	0.3408	0.2414	0.9440

[z]Means with a different letter differ significantly at the 5% level (LSD)
PD (100% petal drop)

Table 33. Effect of 2,4-D on the external quality of 'Palmer' navel orange in the Addo area, South Africa (2008/2009).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	1.39	0.33	1.75	55.56
15 mg·L^{-1} 2,4-D at PD	1.51	0.25	1.23	53.22
20 mg·L^{-1} 2,4-D at PD	1.58	0.54	0.98	52.08
25 mg·L^{-1} 2,4-D at PD	1.54	0.38	1.36	70.83
30 mg·L^{-1} 2,4-D at PD	1.50	0.50	1.47	72.22
P-value	0.9156	0.6260	0.5340	0.4696
Contrast				
Control vs. 2,4-D	0.4841	0.2910	0.5890	0.2110
2,4-D linear	0.8821	0.5785	0.3000	0.1153
2,4-D quadratic	0.5845	0.9473	0.8692	0.4981

Means were separated at the 5% level (LSD)
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
PD (100% petal drop)

Table 34. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Palmer' navel orange in the Addo area, South Africa (2008/2009).

Treatment	Fruit shape[z]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	1.04	9.65	4.99	3.23
15 mg·L^{-1} 2,4-D at PD	1.01	10.86	4.70	3.23
20 mg·L^{-1} 2,4-D at PD	1.04	9.57	4.45	2.98
25 mg·L^{-1} 2,4-D at PD	1.04	9.74	4.87	2.77
30 mg·L^{-1} 2,4-D at PD	1.03	10.56	4.78	2.85
P-value	0.2052	0.8536	0.4610	0.1305
Contrast				
Control vs. 2,4-D	0.3610	0.5973	0.5941	0.2171
2,4-D linear	0.2068	0.8079	0.6458	0.0915
2,4-D quadratic	0.0674	0.4351	0.4209	0.1959

Means were separated at the 5% level (LSD)
[z]Fruit diameter/fruit height
PD (100% petal drop)

Table 35. Effect of 2,4-D on the internal quality of 'Palmer' navel orange in the Addo area, South Africa (2008/2009).

Treatment	^{o}Brix	TA	^{o}Brix:TA	Juice
		--%--		--%--
Control	11.13	1.10	10.17	45.79
15 mg·L^{-1} 2,4-D at PD	10.55	0.95	11.10	47.78
20 mg·L^{-1} 2,4-D at PD	10.75	0.99	10.90	46.91
25 mg·L^{-1} 2,4-D at PD	10.93	1.11	9.98	44.40
30 mg·L^{-1} 2,4-D at PD	10.79	1.07	10.51	46.66
P-value	0.4355	0.2349	0.5084	0.2809
Contrast				
Control vs. 2,4-D	0.3873	0.9623	0.9958	0.7829
2,4-D linear	0.2186	0.1763	0.4230	0.0615
2,4-D quadratic	0.6580	0.7571	0.5529	0.6466

Means were separated at the 5% level (LSD)
PD (100% petal drop)

Table 36. Effect of 2,4-D on the fruit diameter, navel-end size and the percentage of closed navel-ends of 'Autumn Gold' navel orange in the Heidelberg area, South Africa (2008/2009).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navel-ends)	Closed navels
	----------*mm*----------			*--%--*
Control	76.40 d[z]	5.23 a	6.35	19.75 c
15 mg·L^{-1} 2,4-D (ester) at FB	78.85 bc	2.45 b	6.56	60.91 a
25 mg·L^{-1} 2,4-D (ester) at FB	80.59 ab	1.92 b	4.24	54.39 ab
25 mg·L^{-1} 2,4-D (amine) at FB	79.52 abc	2.63 b	6.88	61.64 a
35 mg·L^{-1} 2,4-D (ester) at FB	81.20 a	2.31 b	6.28	61.67 a
25 mg·L^{-1} 2,4-D (ester) at PD	77.35 cd	3.13 b	5.61	40.47 b
P- value	0.0011	0.0002	0.2842	0.0001
Contrast				
Control vs. 2,4-D	0.0013	0.0001	0.6315	0.0001
FB vs. PD	0.0054	0.1295	0.6851	0.0015
2,4-D linear	0.0478	0.8432	0.8150	0.9150
2,4-D quadratic	0.5733	0.4297	0.0399	0.2660
Amine vs. ester	0.3574	0.2905	0.0318	0.3100

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 37. Effect of 2,4-D on the external quality of 'Autumn Gold' navel orange in the Heidelberg area, South Africa (2008/2009).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	2.23	1.11	0.07	5.21
15 mg·L^{-1} 2,4-D (ester) at FB	2.49	1.50	0.01	1.04
25 mg·L^{-1} 2,4-D (ester) at FB	2.39	1.57	0.00	0.00
25 mg·L^{-1} 2,4-D (amine) at FB	2.50	1.61	0.00	0.00
35 mg·L^{-1} 2,4-D (ester) at FB	2.41	1.80	0.13	8.33
25 mg·L^{-1} 2,4-D (ester) at PD	2.48	1.56	0.01	1.04
P- value	0.5710	0.0515	0.3484	0.3076
Contrast				
Control vs. 2,4-D	0.0870	0.0034	0.3972	0.3559
FB vs. PD	0.8033	0.7171	0.5391	0.7048
2,4-D linear	0.6028	0.1590	0.1059	0.0997
2,4-D quadratic	0.6787	0.6331	0.2651	0.2177
Amine vs. ester	0.5030	0.8201	1.0000	1.0000

Means were separated at the 5% level LSD
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
FB (full bloom)
PD (100% petal drop)

Table 38. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Autumn Gold' navel orange in the Heidelberg area, South Africa (2008/2009).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	1.03	12.04 b[z]	4.82 abc	3.11
15 mg·L^{-1} 2,4-D (ester) at FB	1.04	14.65 a	5.22 a	3.15
25 mg·L^{-1} 2,4-D (ester) at FB	1.03	15.38 a	4.99 ab	3.15
25 mg·L^{-1} 2,4-D (amine) at FB	1.03	15.48 a	4.74 bc	3.14
35 mg·L^{-1} 2,4-D (ester) at FB	1.03	15.00 a	4.45 c	3.47
25 mg·L^{-1} 2,4-D (ester) at PD	1.02	14.49 a	4.75 bc	3.11
P- value	0.8294	0.0002	0.0191	0.0887
Contrast				
Control vs. 2,4-D	0.8056	0.0001	0.8665	0.3483
FB vs. PD	0.2169	0.2565	0.1297	0.3100
2,4-D linear	0.6529	0.6160	0.0015	0.0218
2,4-D quadratic	0.6090	0.3639	0.4184	0.1980
Amine vs. ester	0.7522	0.8886	0.2655	0.9385

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
FB (full bloom)
PD (100% petal drop)

Table 39. Effect of 2,4-D on the internal quality of 'Autumn Gold' navel orange in the Heidelberg area, South Africa (2008/2009).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	11.59	1.24	9.87	45.64 a[z]
15 mg·L^{-1} 2,4-D (ester) at FB	11.65	1.28	9.35	38.91 bc
25 mg·L^{-1} 2,4-D (ester) at FB	11.44	1.20	9.61	40.05 b
25 mg·L^{-1} 2,4-D (amine) at FB	11.66	1.22	9.86	40.97 b
35 mg·L^{-1} 2,4-D (ester) at FB	11.50	1.20	9.70	36.97 c
25 mg·L^{-1} 2,4-D (ester) at PD	11.69	1.28	9.17	40.07 b
P- value	0.8602	0.9281	0.8015	0.0001
Contrast				
Control vs. 2,4-D	0.2484	0.9083	0.4682	0.0001
FB vs. PD	0.7215	0.4788	0.3263	0.3912
2,4-D linear	0.7352	0.4481	0.5655	0.1244
2,4-D quadratic	0.7203	0.6480	0.8748	0.0552
Amine vs. ester	0.6123	0.8453	0.6641	0.4612

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 40. Effect of 2,4-D on the fruit diameter, navel-end size, the of percentage closed navel-ends, yield and total fruit number of 'Robyn' navel orange in the Clanwilliam area, South Africa (2007/2008).

Treatment	Fruit diameter	Navel-end size (all fruit)	Navel-end size (open navels)	Closed navels	Yield	Total fruit number
	----------*mm*----------			--*%*--	--*kg/tree*$^{-1}$--	
Control	71.70 c[z]	2.27 bc	4.17	44.05 bcd	132.85	755
20 mg·L^{-1} 2,4-D at FB	71.58 c	1.32 d	4.06	68.12 a	147.17	782
25 mg·L^{-1} 2,4-D at FB	74.29 ab	1.35 d	4.33	67.26 a	125.59	612
20 mg·L^{-1} 2,4-D at PD	71.95 bc	1.71 bcd	3.80	53.37 abc	133.09	711
25 mg·L^{-1} 2,4-D at PD	71.16 c	1.48 cd	3.96	62.16 ab	131.28	710
25 mg·L^{-1} 2,4-D at 2 WAPD	72.48 abc	2.39 b	3.89	35.13 cd	142.73	742
25 mg·L^{-1} 2,4-D at 4 WAPD	74.37 a	3.41 a	5.08	30.68 d	139.08	695
P- value	0.0352	0.0001	0.3337	0.0030	0.8834	0.7065
Contrast						
Control vs. 2,4-D	0.3030	0.3070	0.9572	0.2956	0.7797	0.6166
Timing linear	0.6663	0.0001	0.2218	0.0002	0.3388	0.3605
Timing quadratic	0.0039	0.1389	0.0575	0.9665	0.6982	0.3330
20 mg·L^{-1} vs. 25 mg·L^{-1}	0.2657	0.7366	0.5976	0.6083	0.3355	0.2181

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 41. Effect of 2,4-D on the external fruit quality of 'Robyn' navel orange in the Clanwilliam area, South Africa (2007/2008).

Treatment	Fruit rind colour[z]	Colour at navel-end[y]	Creasing severity[x]	Creasing incidence
				--%--
Control	2.32	1.27	0.40	31.25
20 mg·L^{-1} 2,4-D at FB	2.07	1.70	0.45	32.29
25 mg·L^{-1} 2,4-D at FB	1.97	1.59	0.32	27.08
20 mg·L^{-1} 2,4-D at PD	2.09	1.49	0.50	41.67
25 mg·L^{-1} 2,4-D at PD	1.99	1.32	0.51	35.42
25 mg·L^{-1} 2,4-D at 2 WAPD	2.01	1.44	0.55	46.88
25 mg·L^{-1} 2,4-D at 4 WAPD	1.86	1.72	0.17	16.67
P- value	0.5375	0.2827	0.2826	0.1025
Contrast				
Control vs. 2,4-D	0.0571	0.1129	0.8719	0.7877
Timing linear	0.7055	0.4782	0.4195	0.5374
Timing quadratic	0.6133	0.0788	0.0187	0.0098
20 mg·L^{-1} vs. 25 mg·L^{-1}	0.5081	0.3792	0.6330	0.4253

Means were separated at the 5% level (LSD)
[z]1-8 on CRI colour chart no. 34, 1-orange, 8-green
[y]0-4: 0-orange, 4-green
[x]0-4: 0-no creasing, 4-whole fruit creased
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 42. Effect of 2,4-D on the fruit shape, central axis diameter, rind thickness and pedicel diameter of 'Robyn' navel orange in the Clanwilliam area, South Africa (2007/2008).

Treatment	Fruit shape[y]	Central axis diameter	Rind thickness	Pedicel diameter
		----------*mm*----------		
Control	0.98	11.20 b[z]	5.71 a	3.67 ab
20 mg·L^{-1} 2,4-D at FB	0.97	12.85 a	5.65 a	3.55 bc
25 mg·L^{-1} 2,4-D at FB	0.97	11.97 ab	5.54 ab	3.29 cd
20 mg·L^{-1} 2,4-D at PD	0.98	11.20 b	5.15 bc	3.67 ab
25 mg·L^{-1} 2,4-D at PD	0.98	12.28 ab	4.85 cd	3.85 a
25 mg·L^{-1} 2,4-D at 2 WAPD	0.98	12.64 a	5.36 ab	3.10 d
25 mg·L^{-1} 2,4-D at 4 WAPD	0.99	9.64 c	4.56 d	3.33 cd
P- value	0.2773	0.0001	0.0001	0.0001
Contrast				
Control vs. 2,4-D	0.3456	0.2117	0.0011	0.00453
Timing linear	0.0172	0.0009	0.0003	0.1520
Timing quadratic	0.8093	0.0003	0.7142	0.0925
20 mg·L^{-1} vs. 25 mg·L^{-1}	0.5332	0.8108	0.1477	0.6861

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 43. Effect of 2,4-D on the internal fruit quality of 'Robyn' navel orange in the Clanwilliam area, South Africa (2007/2008).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	9.63	0.96	9.96 bc[z]	48.92 a
20 mg·L^{-1} 2,4-D at FB	9.73	1.01	9.62 c	47.84 a
25 mg·L^{-1} 2,4-D at FB	9.93	0.94	10.45 abc	47.71 a
20 mg·L^{-1} 2,4-D at PD	10.18	0.93	11.02 a	48.54 a
25 mg·L^{-1} 2,4-D at PD	10.48	0.93	11.28 a	45.74 b
25 mg·L^{-1} 2,4-D at 2 WAPD	9.95	0.92	10.77 ab	44.70 b
25 mg·L^{-1} 2,4-D at 4 WAPD	10.27	0.96	10.65 ab	45.53 b
P- value	0.0795	0.3307	0.0263	0.0001
Contrast				
Control vs. 2,4-D	0.0508	0.6984	0.0865	0.0009
Timing linear	0.6173	0.9360	0.9543	0.0057
Timing quadratic	0.5967	0.3009	0.1836	0.0202
20 mg·L^{-1} vs. 25 mg·L^{-1}	0.2427	0.3211	0.1316	0.0156

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (Full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 44. Effect of 2,4-D on the fruit set of ‘Robyn’ navel orange in the Clanwilliam area, South Africa (2007/2008).

Treatment	Fruit set
	--%--
Control	50.33
20 mg·L^{-1} 2,4-D at FB	63.64
25 mg·L^{-1} 2,4-D at FB	60.79
P- value	0.0520
Contrast	
Control vs. 2,4-D	0.0175
2,4-D linear	0.6169

Means were separated at the 5% level (LSD)
FB (Full bloom)

Table 45. Effect of 2,4-D on spot gas exchange measurements ($\mu mol.m^{-2}.s^{-1}$) of 'Navelina' navel orange in the Citrusdal area, South Africa (2008/2009).

Treatment	Net CO_2 assimilation		
	A_{max}	A_{sat}	R_d
Control	5.77	18.39	-3.17
45 $mg \cdot L^{-1}$ 2,4-D at PD	5.70	18.42	-3.29
P- value	0.9257	0.9838	0.6267

PD (100% petal drop)

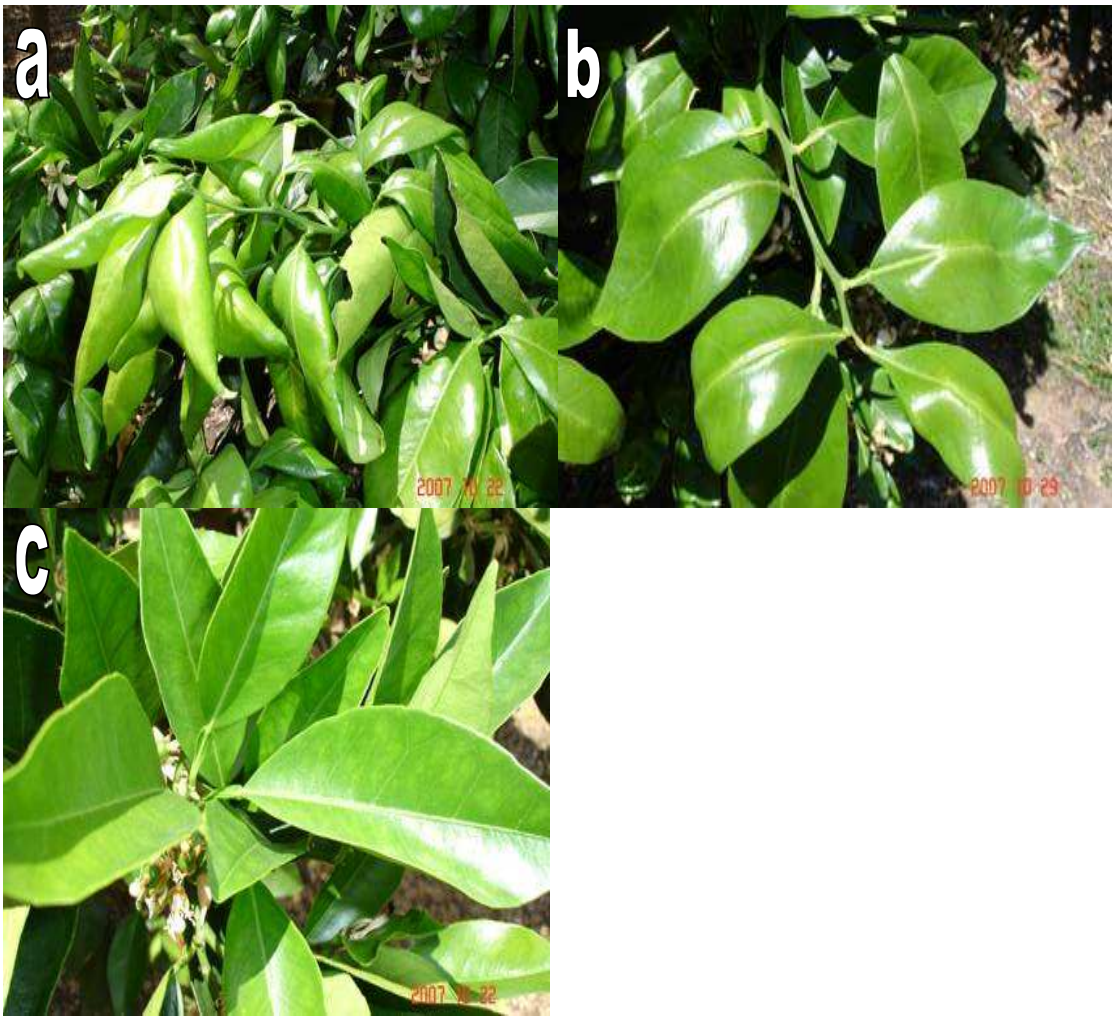


Fig. 1. Effect of 25 mg·L^{-1} (a) and 15 mg·L^{-1} (b) 2,4-D applied at full bloom (FB) on young leaves of the new growth flush of ‘Robyn’ navel orange compared to the untreated control (c).

Fig. 2. Effect of 35 mg·L^{-1} 2,4-D applied at full bloom (FB) on petal drop of ‘Washington’ navel orange (left) compared to the untreated control (right).

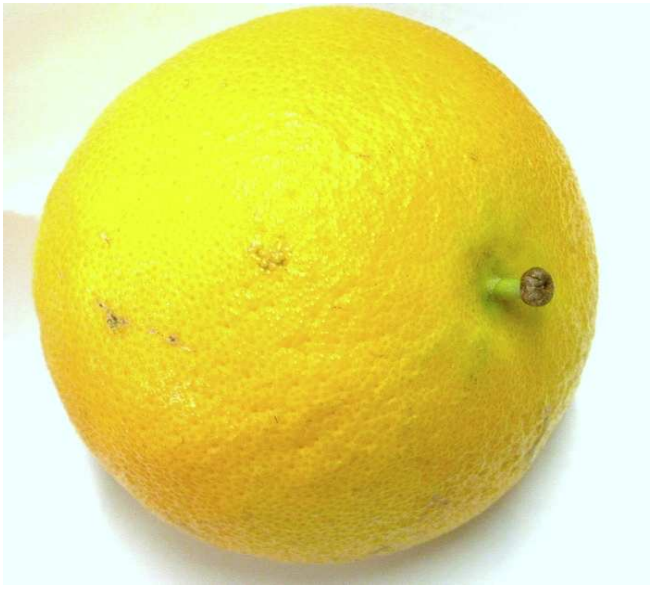
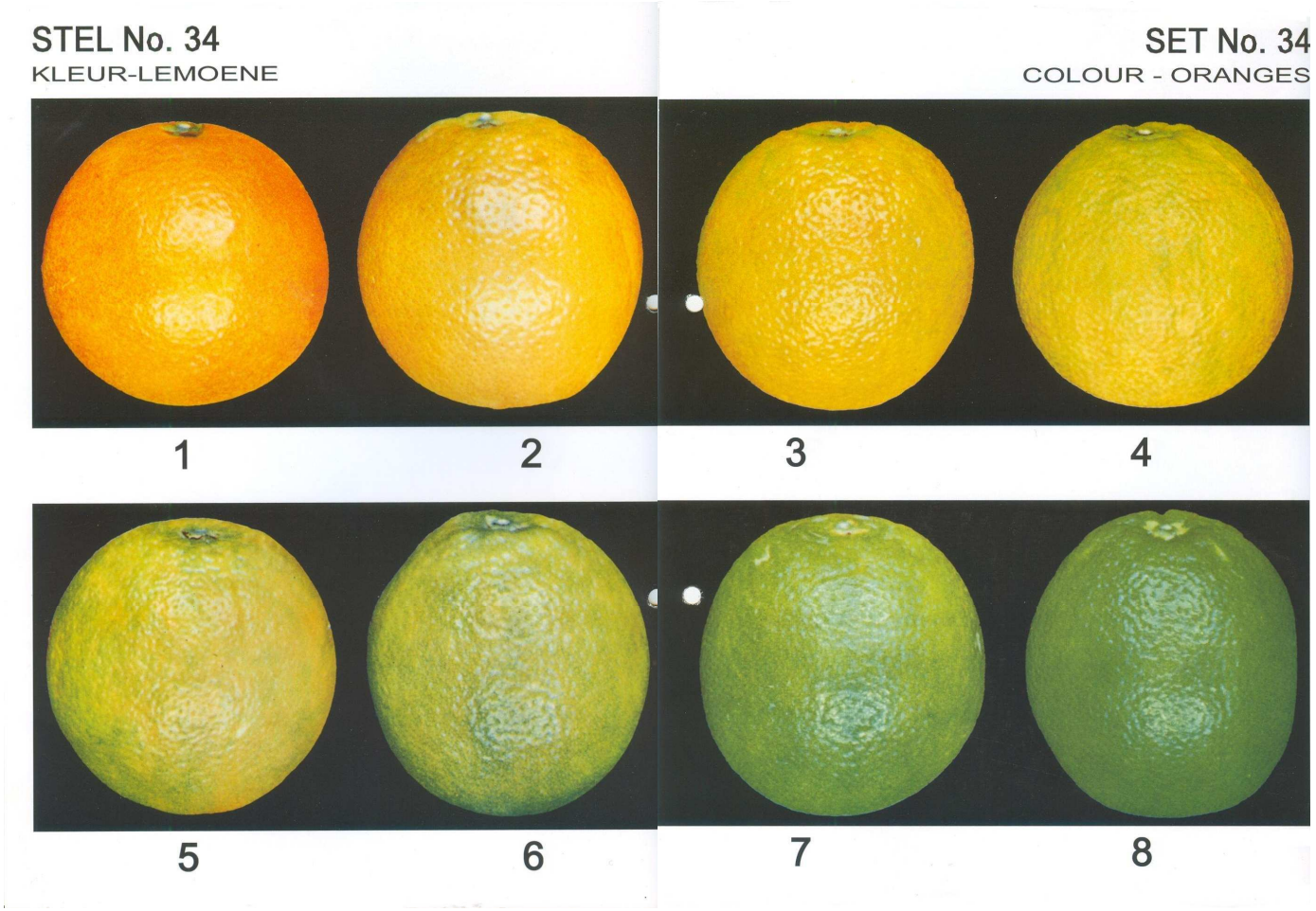
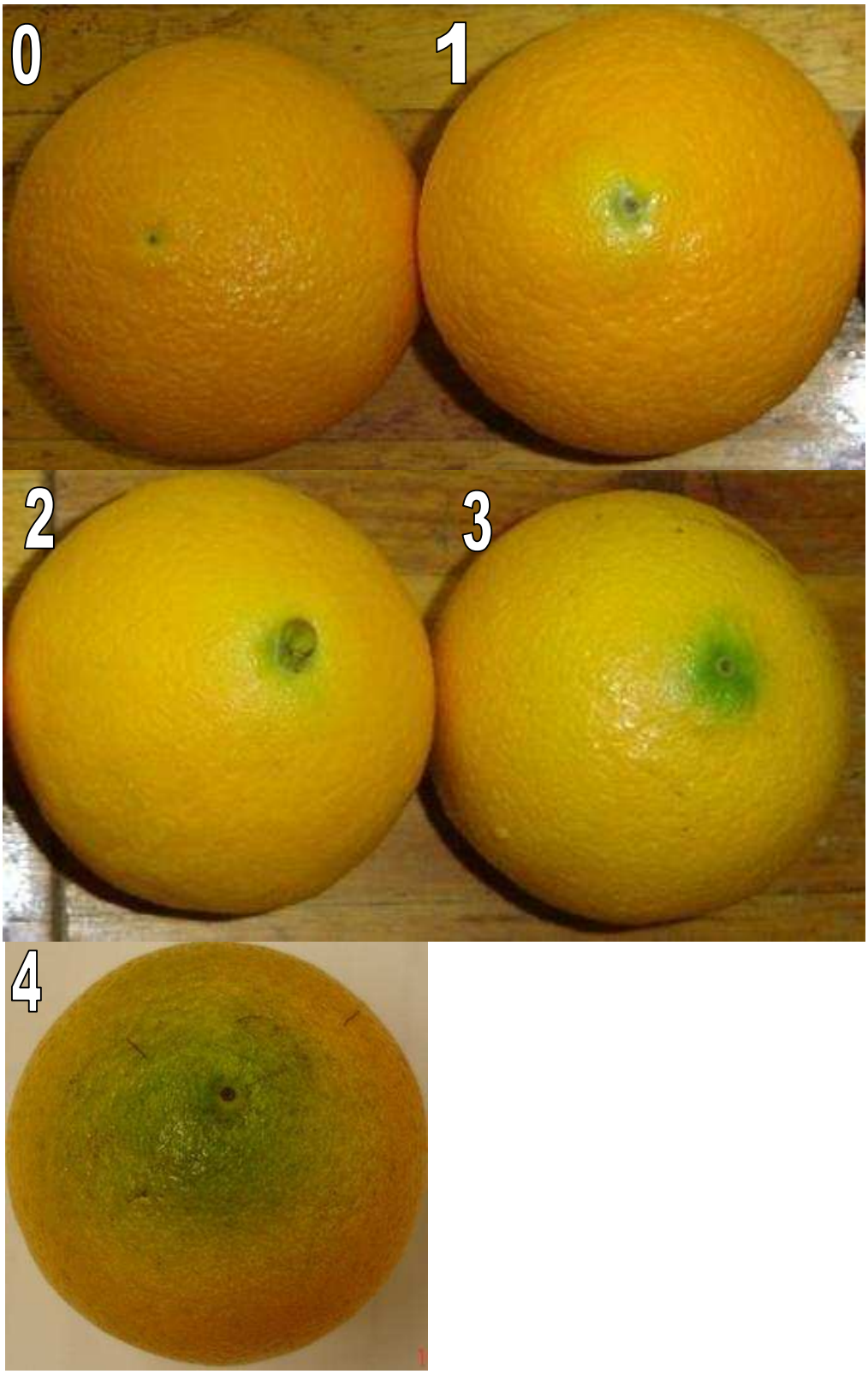


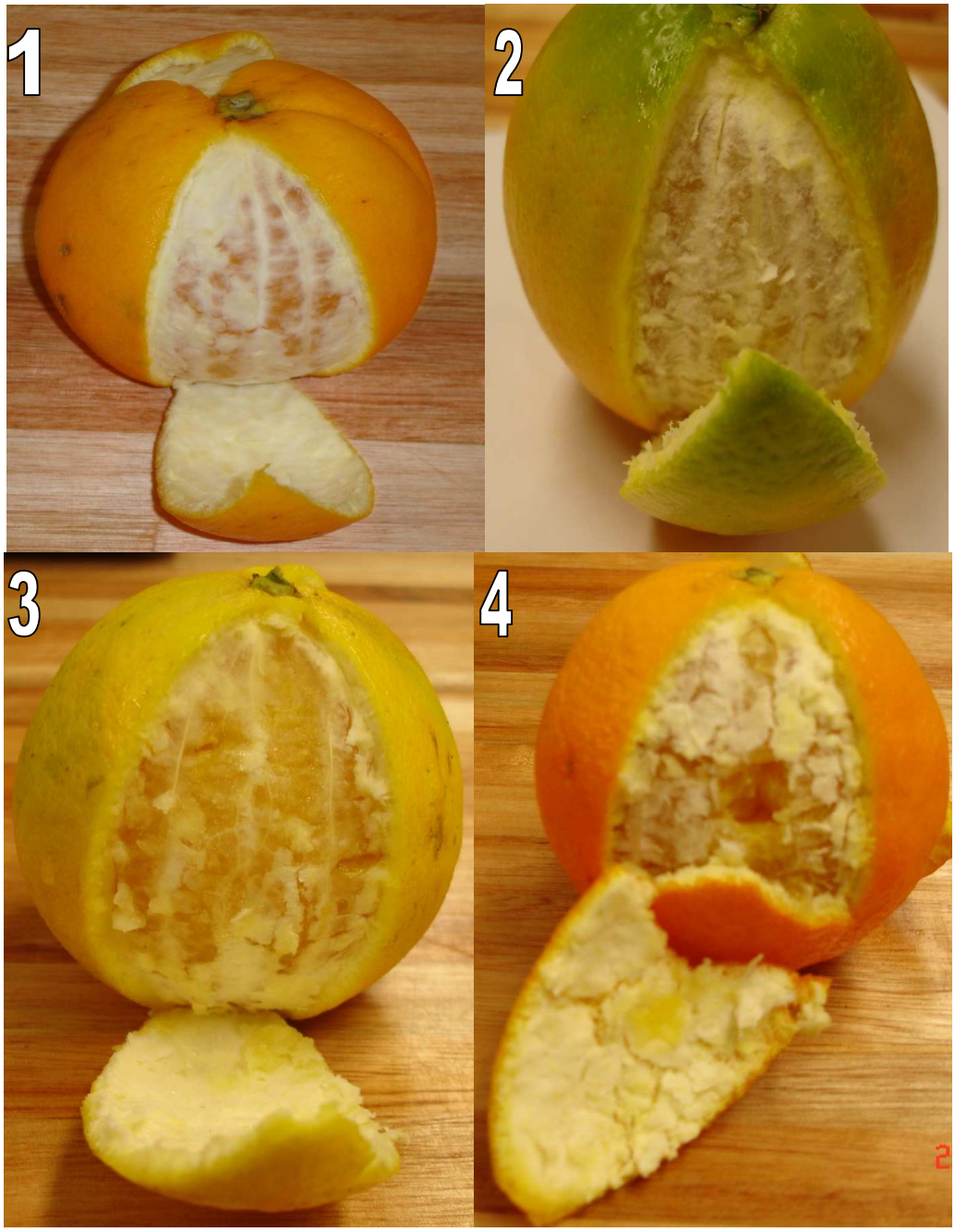
Fig. 3. Effect of 35 mg·L^{-1} 2,4-D applied at full bloom (FB) on the persistence of the style in ‘Autumn Gold’ navel orange fruit at harvest.



Appendix 1. Rind colour rating chart for oranges: 1-orange, 8-green (CRI, 2004).



Appendix 2. Rind colour rating for the navel-end: 0-orange, 4-green.



Appendix 3. Peelabilty rating chart for oranges: (1) easy to peel, rind separates cleanly from the juice segments; (2) relatively easy to peel, bits of the albedo left on the juice segments; (3) relatively difficult to peel, tearing of the juice segments and bits of albedo left on fruit; (4) difficult to peel, tearing of juice segments and rind breaks into pieces during peeling.

PAPER 2: FRUIT GROWTH AND DEVELOPMENT OF THE NAVEL ORANGE [*CITRUS SINENSIS* (L.) OSBECK]: EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON FRUIT MORPHOLOGY

Abstract

Navel oranges have a small secondary fruit located inside the primary fruit at the stylar-end and an opening at the stylar-end called the navel-end opening or the stylar-end aperture. Fruit growth and development was studied in three navel orange cultivars, namely 'Navelina', 'Newhall' and 'Washington' in Citrusdal (32°30'S 19°E), South Africa. The primary fruit diameter, the secondary fruit diameter and the navel-end opening were measured fortnightly using both destructive and non-destructive sampling methods, from physiological fruit drop until fruit maturity. The relationships between the primary fruit, the secondary fruit and the navel opening were studied using correlation analysis on untreated fruit at harvest. In addition, the effect of 2,4-D on fruit morphology, when applied as a treatment to reduce the size of the navel-end opening was also evaluated on the same cultivars. Treatments were applied at full bloom (FB), 100% petal drop (PD), 2 weeks after 100% petal drop (2 WAPD) and 4 weeks after 100% petal drop (4 WAPD), at 15 mg·L^{-1} to 45 mg·L^{-1} of 2,4-D as the iso-octyl ester. The primary fruit and the secondary fruit followed a sigmoidal growth pattern in all the cultivars with a rapid growth phase followed by a declining maturation phase. The navel-end opening developed later, about six weeks after full bloom, but followed a similar growth pattern as the primary fruit and the secondary fruit. The primary fruit size at harvest was not related to the size of the secondary fruit or the navel-end opening. Similarly, the size of the navel-end opening at harvest was not related to the size of the secondary fruit. The primary fruit diameter and height, the secondary fruit diameter and height, the navel-end opening, the primary fruit shape and the secondary fruit shape were not affected by the application of 2,4-D.

Keywords: *navel-end opening; primary fruit; secondary fruit; sigmoidal growth.*

Introduction

Navel oranges [*Citrus sinensis* (L.) Osbeck], have a small secondary fruit located at the stylar-end of the primary fruit (Davies, 1986). The secondary fruit can develop within the primary fruit or may in some cases, protrude outside the primary fruit and may lead to the formation of the navel-end opening or stylar-end aperture (Davies, 1986; Lima and Davies, 1984). Navel oranges are believed to have developed from a mutation of the sweet orange (Davies, 1986).

Morphologically the primary fruit in navel oranges is not different from other sweet oranges and follows a similar developmental pattern (Lima and Davies, 1984). Bouma (1959) and Holtzhausen (1969) studied the development of the primary fruit in 'Washington' navel orange and reported that it followed a sigmoidal pattern with three growth stages, which are similar to those of Valencia sweet orange, as reported by Bain (1958).

The secondary fruit is nearly always present in navel oranges varying in size according to the cultivar and conditions under which the fruit develops (Davies, 1986). Morphologically the secondary fruit is very diverse, ranging from hardly noticeable rind tissue, to well developed fruit that are similar to the primary fruit but only smaller in size (Lima and Davies, 1984). The high auxin levels in navel oranges are thought to be responsible for the development of the secondary fruit (Gustafson, 1939). The secondary fruit also follows the same sigmoidal developmental pattern as the primary fruit (Lima and Davies, 1984).

The navel-end opening is located on the stylar-end of the primary fruit and can vary in size from 0 to 50 mm in diameter (Lima and Davies, 1984). During fruit development the navel-end opening is usually noticeable from about six weeks after anthesis and reaches its final size at fruit maturity (Lima and Davies, 1984). Borders of the navel-end opening and tissues inside the navel-end cavity are made up of the rind of both the primary and secondary fruit (Lima and Davies, 1984).

The synthetic auxin, 2,4-D is used as a plant growth regulator to influence plant growth and development in citrus production, by manipulating key physiological processes both in the orchard and the packhouse (Lovatt, 2005; Stover et al., 2000; Wright, 2004). The main commercial uses of 2,4-D in citrus production are: to increase fruit size (Anthony and Coggins, 1999; Guardiola, 1997), prolong harvest time (Coggins, 1981; Sarooshi, 1982) and postharvest calyx retention (Cronjé et al., 2005; Singh et al., 1977; Wright, 2004). It is used at

low concentrations thereby posing low risk to both man and the environment whilst leaving no hazardous residues (El-Otmani et al., 2000; Monselise, 1979).

The application of 225 mg·L^{-1} 2,4-D during physiological fruit drop increased the height of the primary fruit causing it to become more cylindrical in 'Washington' navel orange (Stewart and Klotz, 1947; Stewart et al., 1951). The treatment also caused the secondary fruit to grow excessively large in size and protrude from the primary fruit (Stewart and Klotz, 1947; Stewart et al., 1951). The application of 20 mg·L^{-1} 2,4-D at full bloom on 'Lane Late' navel oranges reduced the size of the navel-end opening (Gardiazabal, 2006; Saavedra, 2006). Similar results were obtained with 25 mg·L^{-1} 2,4-D applied at 100% petal drop on 'Lane Late', 'Palmer' and 'Robyn' navel oranges (Verreynne, 2008).

The main objective of the research was to study the primary and secondary fruit developmental pattern in the navel orange, as well as the relationship between the primary fruit, the secondary fruit and the navel-end opening. In addition the effect of 2,4-D on the primary fruit, the secondary fruit and the navel-end opening, when applied as treatment to reduce the navel-end opening was evaluated.

Materials and Methods

Plant material

The study was conducted on 'Navelina', 'Newhall' and 'Washington' navel orange trees grafted on Rough lemon rootstock in Citrusdal (32°30'S 19°E), South Africa. The 'Washington' navel trees were planted in 1984 with a tree spacing of 6 m between rows and 6 m within rows and an east-west row direction. The 'Newhall' navel trees were planted in 1993 with a tree spacing of 5 m between rows and 2 m within rows and a north-south row direction. The 'Navelina' navel trees were planted in 1993 with a tree spacing of 5 m between rows and 2 m within rows and a north-south row direction. The study was done in two separate experiments, with the first stage focusing on fruit growth and development and the second on the effect of 2,4-D on fruit morphology.

Experiment 1: Fruit growth and development

Experimental Design

At each site, 8 trees were tagged with buffer trees in between them. These were divided into two blocks of four trees each. Trees were chosen for uniformity in size and only healthy trees were used. All the experiments were carried out in commercial orchards under standard production practices. The trials were conducted in the growing season 2008/2009.

Measurements

On the first block, 10 fruit were tagged on each of the four trees after physiological fruit drop, on all the sides of the tree at the same height from the ground. The primary fruit diameter and navel-end diameter were measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) fortnightly until fruit maturity. The relationship between the final primary fruit diameter and the navel-end opening was determined using correlation and regression analysis on a sample of untreated control fruit taken at harvest in the 2007/2008 and 2009/2008 growing seasons. On the second block of four trees, 10 fruit were randomly picked from each tree after physiological fruit drop fortnightly. The fruit were taken to the lab for destructive measurements. The primary fruit diameter and the navel-end diameter were measured before cutting the fruit into half longitudinally for measurement of the secondary fruit diameter. Fruit growth curves were plotted in SigmaPlot® (version 10.0, Systat Software, Washington, USA). The relationships between the the primary fruit diameter and the navel-end opening diameter were determined using correlation analysis on a sample taken from the untreated control at harvest.

Experiment 2: Effect of 2,4-D on fruit morphology

Treatments

Treatments on 'Navelina' navel orange included an untreated control, 2,4-D applied at 15 $mg{\cdot}L^{-1}$, 25 $mg{\cdot}L^{-1}$, 25 $mg{\cdot}L^{-1}$ + 10 $mg{\cdot}L^{-1}$ gibberellic acid (GA), 35 $mg{\cdot}L^{-1}$ and 45 $mg{\cdot}L^{-1}$ at 100% petal drop (PD). Treatments on 'Newhall' navel orange included an untreated control, 2,4-D applied at 25 $mg{\cdot}L^{-1}$ at full bloom (FB) or PD, 25 $mg{\cdot}L^{-1}$ + 10 $mg{\cdot}L^{-1}$ GA at PD , 25 $mg{\cdot}L^{-1}$ at two weeks after 100% petal drop (2 WAPD) and 25 $mg{\cdot}L^{-1}$ at four weeks after

100% petal drop (4 WAPD). GA was included to improve fruit set. Treatments on 'Washington' navel orange included an untreated control, 2,4-D applied at 15 $mg \cdot L^{-1}$, 25 $mg \cdot L^{-1}$ and 35 $mg \cdot L^{-1}$ at both FB or PD. Only 2,4-D ester (iso-octyl) was applied in all experiments. A non-ionic wetting agent (Break-Thru®) with the active ingredient polyether-polymethylsiloxane-copolymer (1000 $g \cdot L^{-1}$) was added to the spray solution at a rate of 5 ml per 100 L of spray solution in all the experiments. Applications were done using a hand gun sprayer until run-off.

Experimental Design

In the experiment, each treatment consisted of eight single tree replicates in a randomized complete block design with buffer trees between treated trees. Trees were chosen for uniformity in size and only healthy trees were used. All experiments were carried out in commercial orchards under standard production practices. The study was conducted in the 2008/2009 growing season.

Measurements

Six fruit per tree replicate were picked at harvest. The primary fruit diameter and the navel-end opening diameter were measured using an electronic calliper. The fruit were cut into half longitudinally and the secondary fruit diameter was measured. Fruit shape for either primary fruit or secondary fruit was calculated by dividing the respective fruit diameter by the fruit height.

Statistical analysis

Correlation analysis using Pearson product-moment correlation coefficient was carried using PROC CORR (SAS Enterprise Guide 3.02, SAS Institute Inc., Cary, NC, USA). Regression analysis and scatter plots were plotted in Microsoft Office Excel 2003 (Microsoft Corporation, USA). Statistical analysis of variance (ANOVA) was carried out using PROC GLM (version 9.1, SAS Institute Inc., Cary, NC, USA). Mean separation was conducted by least significance difference (LSD) where applicable (P = 0.05) and appropriate contrasts where carried out.

Results

*Experiment 1***.** The primary fruit diameter grew rapidly at first and then slowed down as the fruit neared maturity in all three cultivars (Fig. 1). The non-destructive sampling method done in the field resulted in smoother growth compared to the destructive method in the laboratory. Rapid growth of the fruit occurred due to cell enlargement and was noticeable by the initial steep slope of the graph (December to March). The fruit maturation phase was noticeable from mid-March onwards, with a decreasing fruit growth rate. The secondary fruit diameter followed roughly the same developmental pattern as the primary fruit, although the growth curves were not as smooth due to the destructive sampling method and the high variability in the morphology of the secondary fruit. The rapid growth phase of the secondary fruit, due to cell enlargement, was clearly visible especially in 'Navelina' and 'Newhall' navel orange fruit from December to March. The navel-end opening became noticeable from about 6 weeks after full bloom in December and followed the same growth pattern as the primary fruit and the secondary fruit. The period of cell division with slow growth was visible between the first two measurements in December, followed by the rapid growth rate thereafter due to cell enlargement from mid-December, which occurred later compared to the primary fruit and the secondary fruit. The 'Washington' navel orange had smaller secondary fruit, but larger navel-end openings compared to the other two cultivars.

In all three cultivars, over both seasons, very weak correlations were observed between the primary fruit size and the navel-end opening size at harvest (Figs. 2-4). Although the *P*-values for these correlations were highly significant, the co-efficients of determination showed that only 4% ('Navelina' 2007/2008 season), 7% ('Navelina' 2008/2009 season), 8% ('Newhall' 2007/2008 season), 15% ('Newhall' 2008/2009 season), 4% ('Washington' 2007/2008 season) and 7% ('Washington' 2008/2009 season) of the total variation in the navel-end size could be explained by the primary fruit size at harvest.

A significant correlation between the primary fruit diameter and the navel-end opening in 'Navelina' navel orange at harvest was observed ($r = 0.59$), although only 34% of the variation in navel-end size could be explained by the primary fruit diameter (Table 1). Weak, non-significant correlations between the primary fruit diameter and the secondary fruit diameter ($r = -0.21$) and between the secondary fruit diameter and navel-end opening diameter were observed ($r = -0.08$).

There were no significant correlations between the primary fruit diameter and the navel-end opening diameter and between the secondary fruit diameter and the navel-opening diameter in 'Newhall' navel orange (Table 2). There was a weak, although significant, correlation between the primary fruit diameter and the secondary fruit diameter ($r = 0.34$).

In 'Washington' navel, there were significant correlations between the primary fruit diameter and the navel-end opening diameter ($r = 0.47$), between the primary fruit diameter and the secondary fruit diameter ($r = 0.45$) and between the secondary fruit diameter and the navel-end opening ($r = 0.60$) (Table 3). However, for the strongest correlation ($r = 0.60$), only 36% of the variation in the navel-end opening could be explained by the secondary fruit diameter.

Experiment 2. The application of 2,4-D had no significant effect on the primary fruit diameter and height, the average navel-end size, the secondary fruit diameter and height, the primary fruit shape and the secondary fruit shape of 'Navelina' and 'Washington' navel oranges (Tables 4,6).

The application of 2,4-D had no significant effect on the primary fruit diameter and height, the average navel-end size, the secondary fruit height and the primary fruit shape of 'Newhall' navel oranges (Table 5). The secondary fruit diameter of 'Newhall' navel oranges was reduced by the application of 25 $mg \cdot L^{-1}$ 2,4-D at FB, 2 WAPD and 4 WAPD. Although 25 $mg \cdot L^{-1}$ 2,4-D applied at FB or 2 WAPD affected the secondary fruit shape of 'Newhall' navel orange, there was no clear trend between the treated and the control fruit.

Discussion

Bouma (1959) and Lima and Davies (1984) studied the development of the navel orange fruit and reported that it followed a sigmoidal pattern with three growth stages. In stage 1, growth occurs mainly by cell division and the increase in fruit size is slow. Stage 2 is characterised by cell enlargement and cell differentiation which is accompanied by a rapid increase in fruit size. Stage 3 is marked by a decrease in growth rate, as the fruit matures. The primary fruit followed a similar growth pattern in this study, and stage 2 was clearly visible in all three cultivars from December until mid-March when fruit started to mature, lasting about 14 weeks. Previous studies by Bouma (1959) on 'Washington' navel orange also reported stage 2 of fruit growth to last a similar period of time (Fig 1). The decreasing growth rate in stage 3 was also evident as the fruit maturation coincided with a gradual change in the fruit rind

colour. The pattern of the secondary fruit growth was also similar to that of the primary fruit, concurring with observations by Lima and Davies (1984). The navel-end opening became noticeable about six weeks after full bloom, when the primary fruit was roughly > 20 mm in size. Previous studies by Lima and Davies (1984) also reported a similar later development of the navel-end opening compared to the primary fruit.

The size of the navel-end opening was weakly correlated to the size of the primary fruit at harvest. Only a small percentage of the variation in navel-end size could be explained by the size of the primary fruit which indicates that the size of the primary fruit has little influence on the size of the navel-end opening at harvest. Similarly, there was a weak correlation between the secondary fruit and the nave-end opening, therefore, large secondary fruit will not necessarily mean large navel-end openings. Previous studies by Lima and Davies (1984) also reported a weak relationship between the size of the secondary fruit diameter and the size of the navel-end opening. Furthermore, the primary fruit size and the secondary fruit size were weakly correlated, therefore the size of the primary fruit does not influence the size of the secondary fruit and vice versa.

Full bloom application of 2,4-D is known to reduce the size of the navel-end opening (Verreynne and Mupambi, 2009). The primary fruit diameter and height, the navel-end opening, the secondary fruit diameter and height, the primary fruit shape and the secondary fruit shape were not affected by the application of 2,4-D. Previous studies reported that 2,4-D increased the primary fruit diameter (Anthony and Coggins, 1999; Guardiola, 1997). However, Guardiola (1997) reported that not all cultivars respond to the growth enhancing effect of 2,4-D. The application of 225 $mg \cdot L^{-1}$ 2,4-D increased the primary fruit height, increased the secondary fruit size and caused the primary fruit to become cylindrical in shape (Stewart and Klotz, 1947, Stewart et al., 1951).

In conclusion: the primary fruit, the secondary fruit and the navel-end opening follow a similar developmental pattern although the navel-end opening develops later compared to the primary and the secondary fruit. The primary fruit size has little influence on the size of the secondary fruit and the size of the navel-end opening. Similarly, the size of the navel-end opening is not affected by the size of the secondary fruit. No negative effects were noted on the fruit morphology, mainly due to the low concentrations of 2,4-D used.

Future work should include anatomical studies on 2,4-D treated fruit, using a larger sample size, throughout the growing season to deduce the mode of action of 2,4-D in keeping the navel-end closed. In addition, a study of the factors influencing the size of the navel-end such as the weather after fruit set, abnormal water relations and bearing position of the fruit opening should be carried out.

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Table 1. Relationship between the navel-end opening diameter, the secondary fruit diameter and the primary fruit diameter of untreated 'Navelina' navel orange at harvest in the Citrusdal area, South Africa (2008/2009) (n = 40).

Primary fruit diameter vs. navel-end opening, secondary fruit diameter				
		r	*P*-value	R^2
Primary fruit diameter	Navel-end opening diameter	0.59	0.0001	0.34
Primary fruit diameter	Secondary fruit diameter	-0.21	0.1912	0.04
Secondary fruit diameter	Navel-end opening diameter	-0.08	0.6025	0.01

Table 2. Relationship between the navel-end opening diameter, the secondary fruit and the primary fruit diameter of untreated 'Newhall' navel orange at harvest in the Citrusdal area, South Africa (2008/2009) (n = 40).

Primary fruit diameter vs. navel-end opening, secondary fruit diameter				
		r	*P*-value	R^2
Primary fruit diameter	Navel-end opening diameter	0.18	0.2622	0.03
Primary fruit diameter	Secondary fruit diameter	0.34	0.0319	0.12
Secondary fruit diameter	Navel-end opening diameter	-0.14	0.3761	0.02

Table 3. Relationship between the navel-end opening diameter, the secondary fruit and the primary fruit diameter of untreated 'Washington' navel orange at harvest in the Citrusdal area, South Africa (2008/2009) (n = 40).

Primary fruit diameter vs. navel-end opening, secondary fruit diameter				
		r	*P*-value	R^2
Primary fruit diameter	Navel-end opening diameter	0.47	0.0021	0.22
Primary fruit diameter	Secondary fruit diameter	0.45	0.0038	0.20
Secondary fruit diameter	Navel-end opening diameter	0.60	0.0001	0.36

Table 4. The effect of 2,4-D on the primary fruit diameter, the primary fruit height, the average navel-end size, the secondary fruit diameter, the secondary fruit height, the primary fruit shape and the secondary fruit shape of 'Navelina' navel orange at harvest in the Citrusdal area, South Africa (2008/2009).

Treatment	Primary fruit diameter	Primary fruit height	Navel-end size	Secondary fruit diameter	Secondary fruit height	Primary fruit shape[z]	Secondary fruit shape[y]
	----------*mm*----------						
Control	86.74	92.63	7.95	27.86	36.40	0.94	1.02
15 mg·L^{-1} 2,4-D at PD	83.09	89.42	9.22	25.41	27.76	0.93	0.95
25 mg·L^{-1} 2,4-D at PD	87.49	93.95	10.40	29.04	28.61	0.94	1.05
25 mg·L^{-1} 2,4-D + 10 mg·L-1 GA at PD	85.56	93.34	11.81	27.17	28.75	0.92	0.97
35 mg·L^{-1} 2,4-D at PD	82.63	87.59	9.64	27.96	30.60	0.98	0.94
45 mg·L^{-1} 2,4-D at PD	85.02	90.59	9.23	27.32	29.14	0.94	0.98
P- value	0.5551	0.6265	0.4624	0.7276	0.7073	0.4773	0.3015
Contrast							
Control vs. 2,4-D	0.4548	0.6338	0.1512	0.8577	0.1328	0.8157	0.3927
2,4-D linear	0.9219	0.7411	0.4882	0.5360	0.9773	0.5668	0.8495
2,4-D quadratic	0.6624	0.7729	0.8557	0.2428	0.9110	0.3212	0.4773

Means were separated at the 5% level (LSD)
[z]Primary fruit diameter/primary fruit height
[y]Secondary fruit diameter/secondary fruit height
PD (100% petal drop)

Table 5. The effect of 2,4-D on the primary fruit diameter, the primary fruit height, the average navel-end size, the secondary fruit diameter, the secondary fruit height, the primary fruit shape and the secondary fruit shape of 'Newhall' navel orange at harvest in the Citrusdal area, South Africa (2008/2009).

Treatment	Primary fruit diameter	Primary fruit height	Navel-end size	Secondary fruit diameter	Secondary fruit height	Primary fruit shape[y]	Secondary fruit shape[x]
	----------*mm*----------						
Control	79.64	83.64	7.74	31.78 a[z]	25.91	0.96	1.33 ab
25 mg·L^{-1} 2,4-D at FB	81.22	88.64	4.91	22.78 c	23.57	0.92	0.96 d
25 mg·L^{-1} 2,4-D at PD	81.52	87.15	7.19	29.17 ab	22.35	0.94	1.38 a
25 mg·L^{-1} 2,4-D + 10 mg·L^{-1} GA at PD	85.43	91.20	10.24	27.78 ab	22.75	0.94	1.39 a
25 mg·L^{-1} 2,4-D at 2 WAPD	82.38	87.56	6.50	22.52 c	22.51	0.94	1.05 cd
25 mg·L^{-1} 2,4-D at 4 WAPD	79.35	84.21	10.72	25.44 bc	23.35	0.95	1.16 bc
P- value	0.2606	0.2957	0.0659	0.0006	0.7085	0.6555	0.0001
Contrast							
Control vs. 2,4-D	0.5166	0.2518	0.9626	0.0006	0.0867	0.2242	0.0955
Timing linear	0.4153	0.1833	0.0025	0.9522	0.7451	0.1985	0.2538
Timing quadratic	0.4290	0.7106	0.4062	0.3044	0.6069	0.6739	0.0482

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Primary fruit diameter/primary fruit height
[x]Secondary fruit diameter/secondary fruit height
FB (full bloom)
PD (100% petal drop)
2 WAPD (2 weeks after 100% petal drop)
4 WAPD (4 weeks after 100% petal drop)

Table 6. The effect of 2,4-D on the primary fruit diameter, the primary fruit height, the navel-end size, the secondary fruit diameter, the secondary fruit height, the primary fruit shape and the secondary fruit shape of 'Washington' navel orange at harvest in the Citrusdal area, South Africa (2008/2009).

Treatment	Primary fruit diameter	Primary fruit height	Navel-end size	Secondary fruit diameter	Secondary fruit height	Primary fruit shape[z]	Secondary fruit shape[x]
	----------*mm*----------						
Control	73.22	73.63	6.90	16.95	10.92	1.00	1.66
15 mg·L^{-1} 2,4-D at FB	76.71	77.18	5.04	20.38	13.91	1.00	1.60
25 mg·L^{-1} 2,4-D at FB	77.86	79.57	3.38	20.73	14.07	0.98	1.73
35 mg·L^{-1} 2,4-D at FB	77.90	79.92	3.22	21.31	15.30	0.98	1.48
15 mg·L^{-1} 2,4-D at PD	80.92	79.55	9.2	19.86	14.01	1.02	1.54
25 mg·L^{-1} 2,4-D at PD	78.70	78.46	9.31	19.03	14.30	1.01	1.35
35 mg·L^{-1} 2,4-D at PD	77.84	79.25	12.85	19.21	15.15	0.98	1.31
P- value	0.1786	0.3419	0.1905	0.35.75	0.1513	0.1470	0.1150
Contrast							
Control vs. 2,4-D	0.0134	0.0194	0.9170	0.0328	0.0059	0.8424	0.2302
FB vs. PD	0.2876	0.9586	0.0109	0.1855	0.9799	0.0463	0.0421
2,4-D linear	0.5776	0.6061	0.7466	0.9370	0.2812	0.0294	0.1276
2,4-D quadratic	0.8982	0.9482	0.4145	0.7060	0.6545	0.9776	0.5036
Timing * 2,4-D linear	0.3324	0.5688	0.2543	0.7135	0.9606	0.4727	0.7447
Timing * 2,4-D quadratic	0.7174	0.6161	0.6915	0.8806	0.8749	0.6307	0.2370

Means were separated at the 5% level (LSD)
[z]Primary fruit diameter/primary fruit height
[y]Secondary fruit diameter/secondary fruit height
FB (full bloom)
PD (100% petal drop)

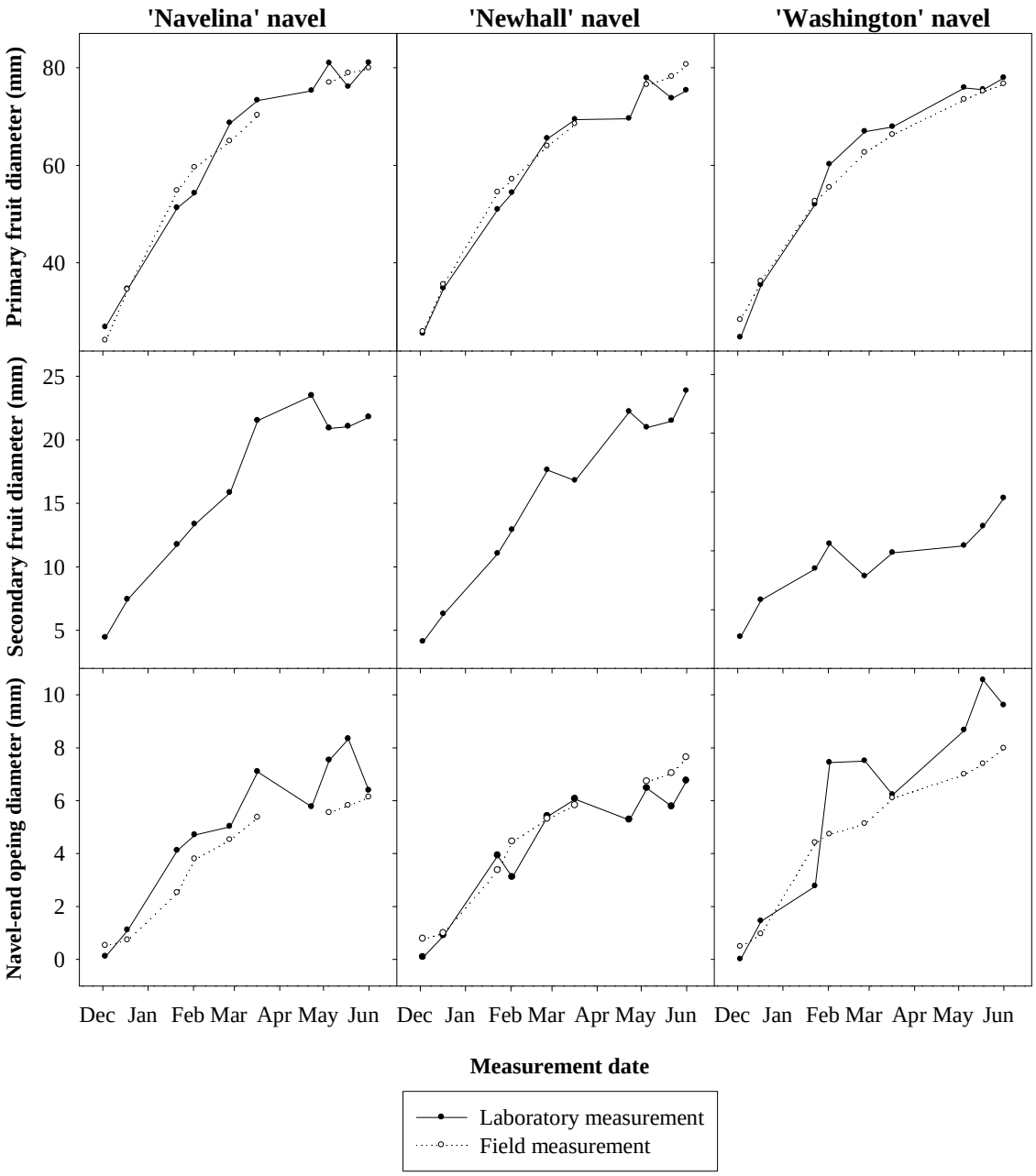

Fig. 1. Growth curves of the primary fruit, the secondary fruit and the navel-end opening in ‘Navelina’, ‘Newhall’ and ‘Washington’ navel orange in the Citrusdal area, South Africa (2008/2009) (n = 40).

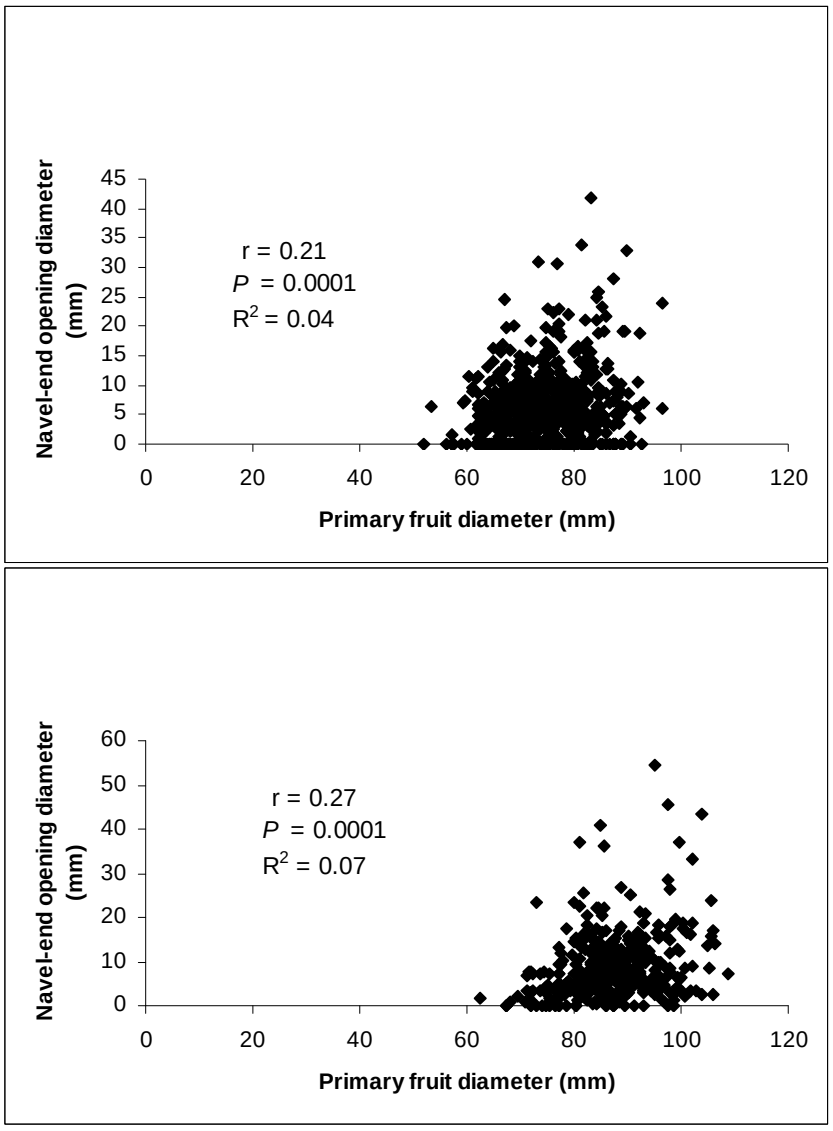

Fig. 2: Relationship between the primary fruit size and the navel-end opening size for untreated 'Navelina' navel orange at harvest in the Citrusdal area, South Africa, in the 2007/2008 season (above) and the 2008/2009 season (below).

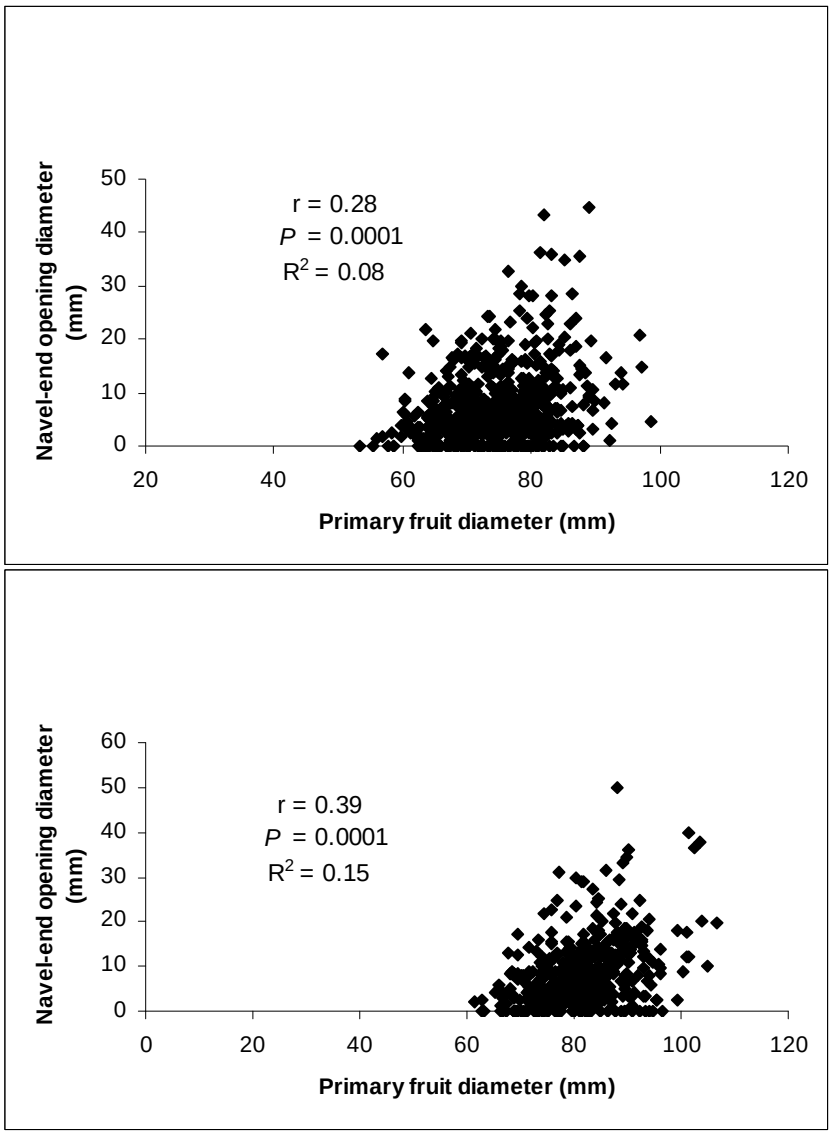

Fig. 3: Relationship between the primary fruit size and the navel-end opening size for untreated 'Newhall' navel orange at harvest in the Citrusdal area, South Africa, in the 2007/2008 season (above) and the 2008/2009 season (below).

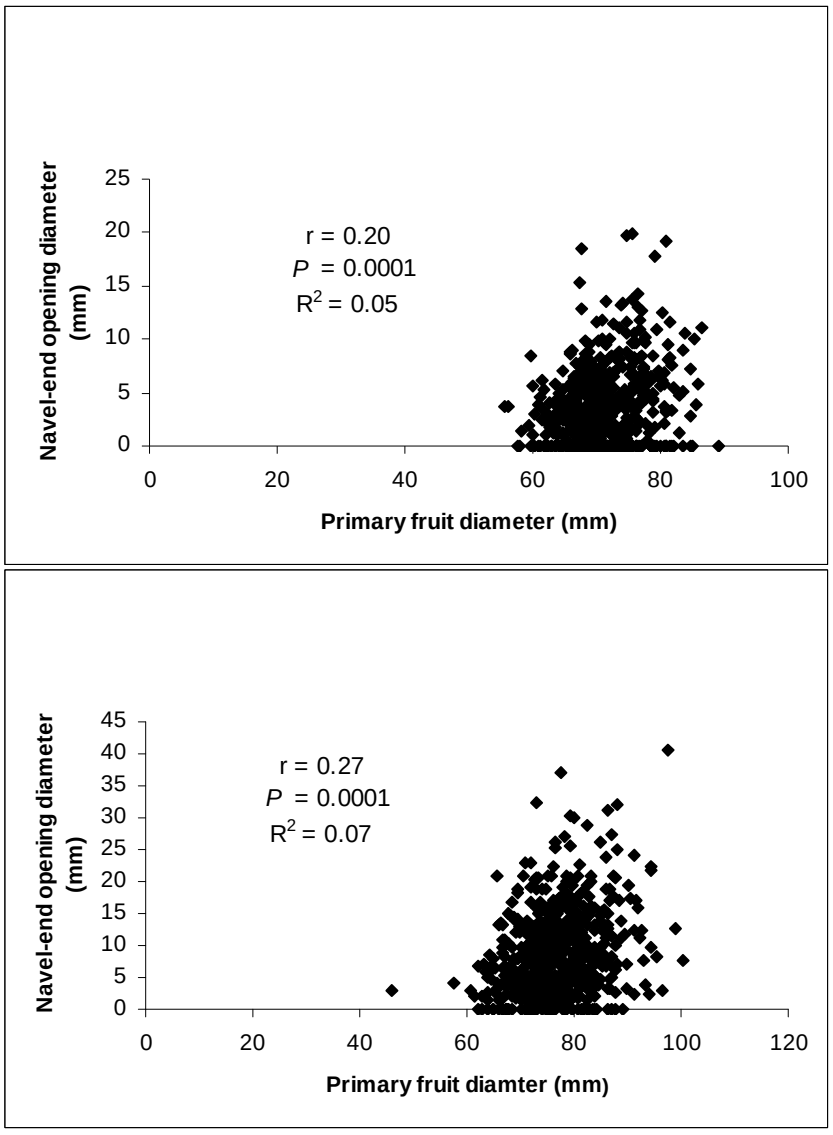

Fig. 4: Relationship between the primary fruit size and the navel-end opening size for untreated 'Washington' navel orange at harvest in the Citrusdal area, South Africa, in the 2007/2008 season (above) and the 2008/2009 season (below).

PAPER 3: THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON FRUIT SPLITTING AND FRUIT QUALITY OF 'MARISOL' CLEMENTINE MANDARIN (*CITRUS RETICULATA*)

Abstract

Fruit splitting is a major physiological disorder of 'Marisol' Clementine mandarin fruit. It develops from the stylar-end of the fruit and causes fruit drop, resulting in yield loss. The effect of 2,4-D on fruit splitting and fruit quality was evaluated in the 2008/2009 growing season. The study was conducted on 'Marisol' Clementine mandarin trees grafted on Troyer citrange rootstock, in Wellington (33°35'S 18°55'E), South Africa. Treatments included an untreated control, 2,4-D applied at 15 mg·L^{-1} or 25 mg·L^{-1} at full bloom (FB) and 15 mg·L^{-1} or 25 mg·L^{-1} at 100% petal drop (PD). Split fruit were removed from the trees and counted every two weeks from mid-March until harvest in early May. At harvest, a sample of 12 fruit per tree was collected to determine internal and external fruit quality. The application of 2,4-D significantly reduced the total number of split fruit collected in all treatments except for 15 mg·L^{-1} at FB. The fruit rind colour, the fruit shape and the rind thickness were not affected by the application of 2,4-D. Internally, the °Brix, the titratable acidity (TA) and the °Brix:TA ratio were not affected by 2,4-D application. However, treated fruit had coarser rinds due to enlarged oil glands and the styles persisted on the treated fruit until fruit maturity. In addition, fruit size was decreased by the treatment with 2,4-D. Therefore, although 2,4-D reduced fruit splitting it cannot be recommended at the timing and concentration applied.

Keywords: *attached styles; external quality; fruit size; internal quality; rind coarseness.*

Introduction

Fruit splitting is a physiological disorder in citrus that develops as a result of cracking of the rind usually from the stylar-end of the fruit (Agusti et al., 2002; Erickson, 1968; Geisel et al., 2001). It occurs in most citrus types, but is more widespread amongst the mandarin cultivars and to some extent in navel oranges (Goldschmidt et al., 1992). Within the mandarin group, it is more severe in mandarin hybrids such as 'Nova' and 'Ellendale' than in Clementine and Satsuma mandarins (Greenberg et al., 2006; Garcia-Luis et al., 2001). Exceptions for the Clementine mandarin subgroup are 'Marisol' and 'Oroval' Clementines which are more prone to splitting (Outspan, 1997). Barry and Veldman (1993) (cited in Barry and Bower, 1997) reported that under South African conditions, 30% split fruit is common in mandarins and sometimes can reach up to 45%. Affected fruit usually drops in the last two to three months before fruit maturity (Garcia-Luis et al., 2001; Goren et al., 1992; Rabe and Van Rensburg, 1996).

Rind resistance to pressure exerted by the expanding pulp and the elasticity of the rind may play a role in determining the severity and incidence of splitting (Garcia-Luis et al., 2001). Hot, dry weather causes the rind to become relatively inelastic making the fruit more liable to split (Sauls, 1995). The morphology of the rind has also been shown to play a role in fruit splitting. The rind is made up of two distinct tissues, the albedo and the flavedo. The albedo is spongy and able to absorb the pressure exerted by the juice vesicles, whilst the flavedo is more rigid and easily cracks under high pressure (Kaufman, 1970). In addition, during fruit growth especially during cell enlargement, the rind often stops growing before the pulp finishes its expansion, leading to fruit splitting (Agusti et al., 2002; Erner et al., 1975). Some evidence of the influence of the rind on fruit splitting has been reported in 'Nova' mandarin where rind thickness and puncturing resistance of the fruit have been negatively correlated with splitting (Almela et al., 1994; Cohen et al., 1972).

Various factors have been linked to fruit splitting in citrus. These include high crop loads, excessive flowering, extreme fluctuations in temperature, humidity, soil moisture and mineral nutrition parameters (Garcia-Luis et al., 2001; Geisel et al., 2001; Rabe and Van Rensburg 1996). Usually, a combination of these factors contributes to fruit splitting, but the extent of the contribution cannot be ascertained as these factors vary from year to year, especially those associated with climate (Geisel et al., 2001). Almela et al. (1994) reported that the percentage of split 'Nova' mandarin fruit varied from 3.8% to 33.4% between years for the same orchard

which might indicate a greater contribution by the climatic factors compared to cultivation practices or orchard condition.

The application of 2,4-D to reduce fruit splitting has produced variable results. Garcia-Luis et al. (2001) reported that a double application of 20 mg·L^{-1} 2,4-D at full bloom and petal drop on the same trees reduced both the number of split fruit per tree and the percentage split fruit in 'Nova' mandarin. Similar results were reported by Almela et al. (1994) using a double application of 2,4-D, two months (June) and one month (July) before the start of the splitting process (northern hemisphere) at 20 mg·L^{-1} on 'Nova' mandarin. In addition, 2,4-D also reduced the percentage of split fruit, but not the number of split fruit per tree in 'Nova' mandarin, when sprayed at an average fruitlet size of 13 mm at 40 mg·L^{-1} (Greenberg et al., 2006). On the other hand, the application of 100 mg·L^{-1} 2,4-D at 100% petal drop or 75% fruitlet drop did not have any effect on fruit splitting of Nova' mandarin (Barry and Bower, 1997). Similar results were also reported by Goren et al. (1992) where 2,4-D was applied at anthesis at 20 mg·L^{-1} on 'Nova' mandarin.

A reduction in fruit splitting will reduce yield loss due to fruit drop caused by fruit splitting. Production costs for the grower might also be decreased as the removal of fruit dropped due to splitting during orchard sanitation will be reduced. The objective of the study was to evaluate the effect of 2,4-D on fruit splitting and fruit quality of 'Marisol' Clementine mandarins.

Materials and Methods

Plant material

The study was conducted on 'Marisol' Clementine mandarin trees grafted on Troyer citrange rootstock in Wellington (33°35'S 18°55'E), South Africa. The orchard was planted in 1996 with a tree spacing of 4 m between rows and 1.75 m within rows and an east-west row direction. Trees were chosen for uniformity in size and only healthy trees were used in the study. The trial was conducted in the growing season of 2008/2009 and the orchard used was under standard commercial practices.

Treatments

The ester form (iso-octyl) of 2,4-D was applied using a hand gun sprayer until run-off. A non-ionic wetting agent (Break-Thru®) with the active ingredient polyether-polymethylsiloxane-copolymer (1000 $g \cdot L^{-1}$) was added to the spray solution at a rate of 5 ml per 100 L of spray solution. Treatments included an untreated control, 2,4-D applied at 15 $mg \cdot L^{-1}$ or 25 $mg \cdot L^{-1}$ at full bloom (FB) and 15 $mg \cdot L^{-1}$ or 25 $mg \cdot L^{-1}$ at 100% petal drop (PD). Each treatment consisted of eight single tree replicates in a randomized complete block design with buffer trees between treated trees.

Measurements

All split fruit were removed from the trees and counted every two weeks from mid-March until harvest in early May. At harvest a sample of 12 fruit per tree was collected to determine internal and external fruit quality. Fruit rind colour was determined based on the no. 36 CRI colour chart for soft citrus [Citrus Research International (CRI), 2004; Appendix 1], with eight being dark green and one fully developed orange colour. Rind coarseness was scored on a scale of 1 to 4 with 1 being a smooth rind and 4 a coarse rind (Appendix 2). The fruit were also evaluated for the presence of attached styles. Fruit diameter, fruit height and pedicel diameter was measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). Fruit shape was determined by dividing the fruit diameter by the fruit height.

Fruit were cut in half longitudinally for internal quality determinations. Rind thickness at the sides of the fruit and at the stylar-end were measured for each fruit using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). The fruit was then juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth and the juice percentage was determined by dividing the weight of the juice by the total fruit weight. ^{o}Brix from the extracted juice was determined using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan). Titratable acidity (TA) expressed as citric acid content was determined by titrating 20 ml of the extracted juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator. The ^{o}Brix:TA ratio was calculated by dividing the ^{o}Brix values by the corresponding TA values.

Statistical analysis

Statistical analysis of variance (ANOVA) was carried out using PROC GLM (version 9.1, SAS Institute Inc., Cary, NC, USA). Mean separation was conducted by least significant difference (LSD) where applicable (P = 0.05) and appropriate contrasts where carried out. Correlation analysis using Pearson product-moment correlation coefficient was carried using PROC CORR (SAS Enterprise Guide 3.02, SAS Institute Inc., Cary, NC, USA).

Results

Fruit splitting

The application of 2,4-D significantly reduced the number of split fruit during the first (2009/03/19) and second (2009/04/02) evaluation dates in all treatments except 15 mg·L^{-1} at FB (Table 1). At the third evaluation date (2009/04/22) there was no significant difference in the number of split fruit per tree between the treated and the control trees (P = 0.4767). All the treatments except 15 mg·L^{-1} at FB spray significantly reduced the total number of split fruit compared to the control. The lowest number of total split fruit was obtained with 15 mg·L^{-1} at PD.

External fruit quality

The application of 2,4-D had no effect on the fruit shape and fruit rind colour (Table 2). Both pedal drop applications of 2,4-D resulted in significantly coarser rinds and enlarged oil glands compared to the control fruit (Fig. 1, Table 3). All the 2,4-D treatments caused the styles to persist on the fruit until maturity (Fig. 2). The lower concentration of 2,4-D (15 mg·L^{-1}) at both PD and FB resulted in a higher percentage of fruit with styles attached than the higher concentration (25 mg·L^{-1}) (Table 2).

Fruit size, rind thickness and pedicel diameter

Fruit sampled from the treated trees were significantly smaller than the control fruit (Table 3). The application of 2,4-D had no effect on the rind thickness at the equatorial region and the bottom of the fruit. There was no clear trend in pedicel diameter due to the treatment with 2,4-D (Table 3).

Internal fruit quality

There were no significant differences in the ^{o}Brix, TA, the ^{o}Brix:TA ratio and the juice content (%) between treated and control fruit (Table 4).

In addition, there was a weak relationship between rind thickness at the bottom of the fruit (PTB) and fruit splitting (Table 5). In contrast, a very strong negative correlation between fruit splitting and the percentage of styles attached was observed (r = -0.90).

Discussion

The application of 2,4-D reduced fruit splitting in 'Marisol' Clementine fruit. The reduction in fruit splitting by 2,4-D has been reported previously by Almela et al. (1994), Garcia-Luis et al. (2001) and Greenberg et al. (2006). The incidence of fruit splitting throughout the season was reduced as fruit neared maturity. This has also been previously reported by Rabe and Van Rensburg (1996). The application of 2,4-D had no effect on the fruit shape, which is known to affect fruit splitting in 'Nova' mandarin with oblate fruit being more prone to split (Garcia-Luis et al., 2001). Fruit rind colour was not affected by the treatments, concurring with results reported on 'Washington' navel orange by Stewart et al. (1951). The application of 2,4-D affected rind coarseness. The same effect has been reported in oranges by Stewart and Klotz (1947), but at much higher concentrations (225 $mg \cdot L^{-1}$). The application of 2,4-D has not been reported to affect the rind coarseness in mandarins before. The rind of 'Marisol' Clementine is naturally coarse with well defined oil glands (Outspan, 1997). The increase in rind coarseness is caused by outward and inward elongation of the oil glands (Stewart and Klotz, 1947) as observed in this study.

The application of 2,4-D caused the styles to persist on the fruit until maturity, which was previously reported on navel oranges by Krezdorn (1969). Attached styles might damage other fruit after harvesting which could lead to quality loss and decay. The application of 2,4-D caused a reduction in fruit size, which is a concern since small fruit size is already a problem in the mandarin group (El-Otmani et al., 1996; Guardiola et al., 1988). However, results are contradictory to previous reports where 2,4-D increased fruit size in 'Esbal' Clementine (Duarte et al., 1996; Guardiola and Garcia-Luis, 2000).The application of 2,4-D had no effect on the equatorial (measured on the side) rind thickness. Previous work on mandarins has produced variable results with Garcia-Luis et al. (2001) reporting an increase

in rind thickness whilst Almela et al. (1994) reported no effect on rind thickness. Rind thickness at the bottom of the fruit (stylar-end) was not affected in our study. In contrast 2,4-D increased rind thickness at the stylar-end in 'Nova' mandarin (Garcia-Luis et al., 2001). The treatments had no effect on the ^{o}Brix, TA, the juice content (%) and the ^{o}Brix:TA ratio. Similar results were reported by Duarte et al. (1996) on 'Esbal' Clementine.

There was no relationship between the rind thickness at the bottom of the fruit and fruit splitting. An inverse relationship between rind thickness at the bottom of the fruit and fruit splitting has been reported previously on 'Nova' mandarin (Almela et al., 1994). A strong negative correlation between the number of attached styles at harvest and fruit splitting was observed and the 2,4-D treatment caused the styles to persist on the fruit for a longer period of time. Continued attachment of the style on the fruit might reduce fruit splitting by keeping the stylar-end of the fruit intact for a longer time therefore preventing it from cracking open.

Although 2,4-D significantly reduced fruit splitting in this study, it cannot be recommended commercially at the concentrations and timings evaluated. Future research should include the use of lower concentrations of 2,4-D to attempt to reduce the coarse rinds with enlarged oil glands and should include other split-prone mandarin cultivars such as 'Nova' and 'Ellendale'.

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Table 1. Effect of 2,4-D on the number of split fruit on 'Marisol' Clementines trees in the Wellington area, South Africa (2008/2009).

Treatment	Number of split fruit per tree per evaluation date (2009)			Total number of split fruit per tree
	19 March	2 April	22 April	
Control	9.50 a[z]	3.63 a	2.13	15.25 a
15 mg·L^{-1} 2,4-D at FB	5.13 ab	2.75 ab	1.88	9.75 ab
25 mg·L^{-1} 2,4-D at FB	4.63 b	1.25 bc	1.00	6.83 b
15 mg·L^{-1} 2,4-D at PD	1.75 b	0.50 c	1.88	3.13 b
25 mg·L^{-1} 2,4-D at PD	4.13 b	0.88 bc	1.50	6.50 b
P-value	0.0434	0.0326	0.4767	0.0192
Contrast				
Control vs. 2,4-D	0.0058	0.0120	0.2124	0.0034
FB vs. PD	0.2570	0.0952	0.6640	0.1599
2,4-D linear	0.5800	0.4653	0.8279	0.9186
Timing * 2,4-D linear	0.3979	0.2276	0.1985	0.2079

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 2. The effect of application of 2,4-D on the external fruit quality of 'Marisol' Clementines trees in the Wellington area, South Africa (2008/2009).

Treatment	Fruit shape[y]	Fruit rind colour[x]	Rind coarseness[w]	Fruit with style attached
				--%--
Control	1.06	4.93	1.66 c[z]	0.00 c
15 mg·L^{-1} 2,4-D at FB	1.06	5.03	1.76 abc	40.63 ab
25 mg·L^{-1} 2,4-D at FB	1.04	5.15	1.73 bc	34.56 b
15 mg·L^{-1} 2,4-D at PD	1.02	5.34	2.03 a	50.00 a
25 mg·L^{-1} 2,4-D at PD	1.02	5.32	1.97 ab	32.29 b
P-value	0.0549	0.5446	0.0433	0.0001
Contrast				
Control vs. 2,4-D	0.1213	0.2244	0.0603	0.0001
FB vs. PD	0.0245	0.2240	0.0120	0.4866
2,4-D linear	0.2166	0.8086	0.6144	0.0255
Timing * 2,4-D linear	0.3905	0.7312	0.8870	0.2574

[z]Means with a different letter differ significantly at the 5% level (LSD)
[y]Fruit diameter/fruit height
[x]1-8: on colour chart, 1-orange, 8-green
[w]1-4: 1-smooth rind, 4 coarse rind
FB (full bloom)
PD (100% petal drop)

Table 3. Effect of 2,4-D on fruit size, rind thickness and pedicel diameter of 'Marisol' Clementines trees in the Wellington area, South Africa (2008/2009).

Treatment	Fruit size	Rind thickness		Pedicel diameter
		Equatorial region	Bottom of the fruit	
	----------*mm*----------			
Control	57.89 a[z]	2.71	2.61	3.38 b
15 mg·L^{-1} 2,4-D at FB	53.78 b	2.37	2.67	2.88 c
25 mg·L^{-1} 2,4-D at FB	52.60 b	2.50	2.58	3.50 ab
15 mg·L^{-1} 2,4-D at PD	51.49 b	2.69	3.09	3.75 a
25 mg·L^{-1} 2,4-D at PD	53.09 b	2.43	2.31	3.22 bc
P-value	0.0030	0.1176	0.0592	0.0005
Contrast				
Control vs. 2,4-D	0.0002	0.0852	0.7814	0.7370
FB vs. PD	0.4056	0.2441	0.6758	0.0207
2,4-D linear	0.8530	0.5611	0.0198	0.7326
Timing * 2,4-D linear	0.2057	0.0860	0.0569	0.0001

[z]Means with a different letter differ significantly at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 4. Effect of 2,4-D on the internal fruit quality of 'Marisol' Clementines trees in the Wellington area, South Africa (2008/2009).

Treatment	°Brix	TA	°Brix:TA	Juice
		--%--		--%--
Control	10.79	1.21	9.00	42.32
15 mg·L^{-1} 2,4-D at FB	10.96	1.21	9.17	42.06
25 mg·L^{-1} 2,4-D at FB	11.08	1.25	8.99	40.75
15 mg·L^{-1} 2,4-D at PD	10.71	1.28	8.62	38.61
25 mg·L^{-1} 2,4-D at PD	10.49	1.23	8.73	39.80
P-value	0.5154	0.5719	0.6419	0.3293
Contrast				
Control vs. 2,4-D	0.9380	0.4314	0.6826	0.2127
FB vs. PD	0.1043	0.5419	0.1590	0.1309
2,4-D linear	0.8232	0.9291	0.9035	0.9644
Timing * 2,4-D linear	0.5042	0.1748	0.6144	0.3836

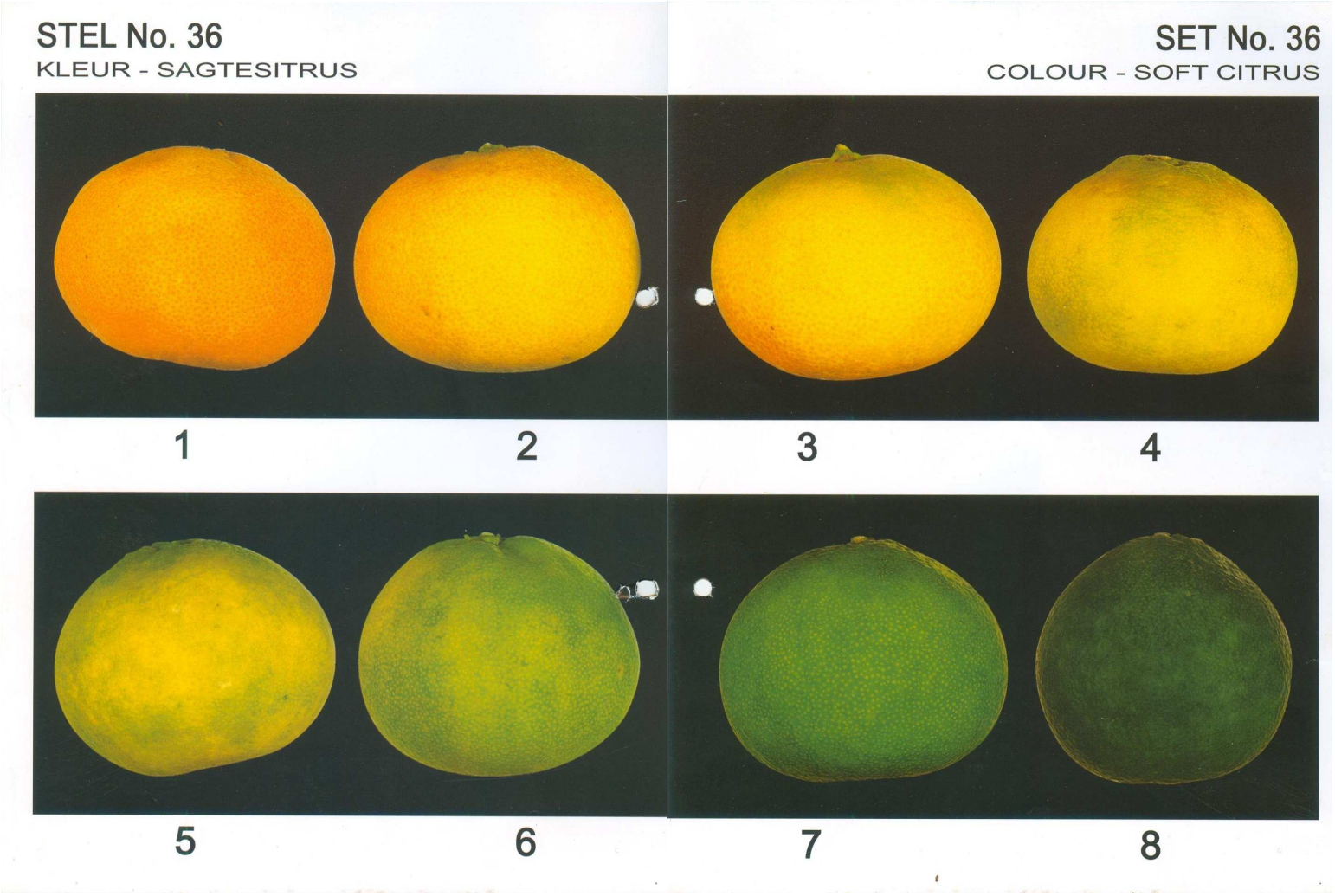
Means were separated at the 5% level (LSD)
FB (full bloom)
PD (100% petal drop)

Table 5. Relationship between the rind thickness at the bottom of the fruit (RTB) or the percentage of styles attached and fruit splitting of 'Marisol' Clementine fruit in the Wellington area, South Africa (2008/2009).

Fruit splitting vs. RTB or the percentage of styles attached				
			r	*P*-value
Fruit splitting	vs.	RTB	-0.38	0.0144
Fruit splitting	vs.	Percentage of styles attached	-0.90	0.0402

Fig. 1. Effect of 15 mg·L^{-1} 2,4-D applied at 100% petal drop (PD) (left) on the oil glands and rind coarseness of ‘Marisol’ Clementine fruit compared to the untreated control (right).

Fig. 2. Effect of 25 mg·L^{-1} 2,4-D applied at 100% petal drop (PD) on the persistence of the style in ‘Marisol’ Clementine fruit at harvest.



Appendix 1. Rind colour rating chart for soft citrus (CRI, 2004).

Appendix 2.Rind coarseness rating chart for soft citrus.

5. OVERALL DISCUSSION AND CONCLUSION

Fruit with large open navel-ends are predisposed to splitting and navel-end rot in the orchard and are also culled in the packhouse reducing the export packout. The synthetic auxin 2,4-D was applied to reduce the size of the navel-end opening on six different navel orange cultivars, using different timings (full bloom to 4 weeks after 100% petal drop) and concentrations (15 to 45 $mg \cdot L^{-1}$). The application of 2,4-D at full bloom (FB) increased the percentage of closed navel-ends (by up to 42%) and reduced the average navel-end size of all the fruit sampled (by up to 5 mm), in all the cultivars and the different production regions, over both seasons, regardless of the concentration applied. The average navel-end size of only the fruit with open navel-ends was not affected, therefore 2,4-D seems to close the navel-end opening completely, rather than making it smaller. Later applications at 100% petal drop (PD), as well as 2 weeks (2 WAPD) and 4 weeks after 100% petal drop (4 WAPD) were generally ineffective. The yield and total fruit number per tree were not affected by the treatments.

There were no major negative side effects on external and internal fruit quality except for the reduction in juice content (%) especially with the PD and later applications. The postharvest storage quality of the fruit was not affected by the treatments. The application of 2,4-D damaged the young leaves on new growth flushes, but had no effect on their photosynthetic capacity. Leaf damage due to 2,4-D was more pronounced at higher concentrations and barely visible at the lowest concentration (15 $mg \cdot L^{-1}$). Stylar abscission was delayed by the FB application of 2,4-D, which most likely plays a role in the mode of action in which 2,4-D keeps the navel-ends closed. Normally, the enzyme cellulase breaks down the vascular bundles connecting the style to the fruit leading to its abscission. The application of 2,4-D might decrease fruit ethylene, levels which in turn could suppress cellulase activity and keep the style intact. However, more anatomical work needs to be done to find the exact mode of action. The application of 15 $mg \cdot L^{-1}$ 2,4-D at FB can be recommended to increase the percentage of closed navel-ends and possibly increase export packouts.

Navel oranges have a small secondary fruit located inside the primary fruit at the stylar-end and an opening at the stylar-end called the navel-end opening or the stylar-end aperture. The primary fruit and the secondary fruit followed a sigmoidal growth pattern with a rapid growth phase followed by a declining maturation phase in all the cultivars. The navel-end opening developed later, starting about six weeks after FB, but followed a similar growth pattern as the

primary fruit and the secondary fruit. The primary fruit size at harvest was not related to the size of the secondary fruit or the navel-end opening. Similarly, the size of the navel-end opening at harvest was not related to the size of the secondary fruit. The primary fruit diameter and height, the secondary fruit diameter and height, the navel-end opening, the primary fruit shape and the secondary fruit shape were not affected by the application of 2,4-D.

Fruit splitting is a major physiological disorder of 'Marisol' Clementine mandarin fruit. The application of 2,4-D significantly reduced the total number of split fruit collected in all treatments except for 15 $mg \cdot L^{-1}$ at FB. The fruit rind colour, the fruit shape and the rind thickness were not affected by the application of 2,4-D. However, treated fruit had coarser rinds due to enlarged oil glands and the styles persisted on the treated fruit until fruit maturity. In addition, fruit size was decreased by the treatment with 2,4-D. Internally, the ^{o}Brix, the titratable acidity (TA) and the ^{o}Brix:TA ratio were not affected by 2,4-D application. Therefore, although 2,4-D reduced fruit splitting it cannot be recommended at the timing and concentration applied. Further studies should include lower concentrations of 2,4-D, later treatments and other split prone cultivars.