Fruit split and fruit size studies on Citrus

by

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DECLARATION

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SUMMARY: Fruit split and fruit size studies on citrus

Fruit size and the integrity of the rind are key components that determine the value of a citrus fruit. The application of 2,4-dichlorophenoxy acetic acid (2,4-D) to reduce splitting, a physiological disorder which entails cracking of the rind as well as to increase fruit size was conducted on three different split-susceptible mandarin and two split-susceptible orange cultivars. Treatments were applied directly after the physiological fruit drop period, as well as in January and February at 10 $mg \cdot L^{-1}$, alone or in combination with calcium (Ca), potassium (K) or gibberellic acid (GA_3). Application of 2,4-D directly after physiological fruit drop, either alone or in a tank-mix with K, consistently reduced the number of split mandarin fruit, with later applications in January and February generally being ineffective. Post physiological fruit drop application of 10 $mg \cdot L^{-1}$ 2,4-D significantly increased growth rate ($mm.day^{-1}$) of all the mandarin cultivars, resulting in increased fruit size. Differences in sensitivity of cultivars to 2,4-D were evident, with the January application reducing the splitting in 'Midknight' Valencia. However, all the 2,4-D treatments reduced the fruit growth rate of the orange cultivars. The 2,4-D treatments, in terms of splitting, increased rind thickness, -strength and -coarseness of 'Marisol' Clementine, throughout fruit development. In addition fruit diameter and –length increased to such an extent that the fruit shape was altered (reduced d/l-ratio), reducing the potential of the rind to crack and the fruit to split, however rind coarseness of treated fruit was also increased. There were no major negative side effects on internal and external fruit quality, except for a possible reduction in juice content (%). Therefore, 10 $mg \cdot L^{-1}$ 2,4-D can be applied directly after physiological fruit drop on 'Marisol' Clementine and 'Mor' mandarin to reduce fruit splitting.

OPSOMMING: ’n Studie oor vrugsplit en -grootte van sitrusvrugte

Vruggrootte asook die integriteit van die skil is belangrike aspekte in die bepaling van ’n sitrusvrug se waarde. Die toediening van 2,4-dichlorofenoksie asynsuur (2,4-D) om vrugsplit, 'n fisiologiese defek wat tot die kraak van die sitrusskil lei, te verminder is getoets op drie mandaryn- en twee lemoenkultivars. Hiermee saam is die potensiaal van 2,4-D om vruggrootte te verbeter ook geëvalueer. Die 2,4-D behandelings is direk na die fisiologiese vrugval periode toegedien, asook in Januarie en Februarie, teen 10 $mg{\cdot}L^{-1}$, alleen of in kombinasie met kalsium (Ca), kalium (K) of gibberelliensuur (GS_3). Al die mandarynkultivars het ’n vermindering in die totale aantal gesplete vrugte getoon indien die 2,4-D (enkel of in kombinasie met K) toegedien was direk na fisiologiese vrugval. Suksesvolle behandelings het ook 'n toename in vruggrootte tot gevolg gehad. Toediening van behandelings in Januarie en Februarie was oor die algemeen oneffektief. Verskille in kultivar sensitiwiteit teenoor 2,4-D is gevind, met vrugsplit in ‘Midknight’ Valencia wat verminder was deur die Januarie toediening van 2,4-D. Al die 2,4-D behandelings het vruggrootte van die lemoenkultivars verlaag. Daar is bevind dat die 10 $mg.L^{-1}$ 2,4-D, enkel of in kombinasie met K, ‘n toename in beide skildikte en –sterkte van ‘Marisol’ Clementine teweeg bring asook ’n growwer skil. Behandelings met 2,4-D het vrugdeursnee en –lengte laat toeneem, wat ’n verandering in vrugvorm tot gevolg gehad het, tot so ’n mate dat vrugte minder geneig was om gesplete te wees. Behalwe vir ’n moontlike verlaging in die sapinhoud (%) van vrugte, was daar geen noemenswaardige negatiewe effekte op interne en eksterne vrugkwaliteit nie. Die toediening van 10 $mg.L^{-1}$ 2,4-D direk na fisiologiese vrugval kan dus aanbeveel word op mandaryn kultivars wat geneig is tot vrugsplit.

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1. GENERAL INTRODUCTION

Citrus fruit splitting is a physiological disorder in Clementine mandarin, mandarin hybrids as well as 'Navel' and 'Valencia' orange and is a consequence of micro-cracks developing at the stylar-end of the fruit rind. As the fruit matures, the split lesion extends towards the equatorial region of the fruit and eventually leads to premature drop. In severely affected orchards, fruit losses of as much as 30% have been reported (Barry and Bower, 1997).

Citrus fruit growth follows a sigmoïdal curve and consists of three development stages, as described by Bain (1958). Stage I is characterized by cell division, with the total volume of a single fruitlet predominantly consisting of the rind. Stress factors in this period, such as nutritional imbalances, water deficit, high flower number and fruit set hampers sufficient cell division and lead to the development of a weak and thin rind. During stage II, increase in fruit volume occurs due to cell enlargement of the pulp and physical splitting of the citrus fruit becomes visible. During stage III, the fruit starts to ripen, very little or no increase in fruit volume occurs and split fruit drop from the tree.

Not only does splitting lead to unwanted reduction in crop load, but also require additional labour to sanitize orchards as split fruit provide perfect conditions for manifestation of insect pest and decay. A commercial solution is therefore required to control fruit splitting as it occurs throughout South African production regions. Most of the studies on the control of fruit splitting focussed on increasing the rind thickness and -strength of split-prone species by applying pre-harvest mineral nutrient sprays to the canopy or plant growth regulator (PGR) foliar sprays. Although results were erratic, PGR application of the synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D) were generally more successful.

In this study the treatment effect on splitting of different combinations of 10 mg·L^{-1} 2,4-D and calcium (Ca) and potassium (K) were evaluated in different split-prone cultivars in their different localities, over two production seasons. In addition to splitting, the effect of different timings of 10 mg·L^{-1} 2,4-D applications, starting after physiological fruit drop, on fruit size and general fruit quality parameters was also evaluated.

Synthetic auxins have a direct stimulatory effect on fruit growth and size. The isopropylester formulation of 2,4-D is registered as a fruit size enhancer in California. It is rapidly absorbed by roots, stems and leaves and translocated in the phloem to young meristematic tissue (Ashton et al., 1991). It accumulates in organs such as young leaves, flowers or fruitlets where it increases sink strength of these organs by stimulating cell expansion (Mitchell, 1961). However, except for difference in cultivar sensitivity to synthetic auxins, success of application is dependent on timing, as well as concentration and application rate.

In this study the effect of different timings of 2,4-D treatments on a variety of cultivars was evaluated, with the aim of providing producers with a commercial, viable solution to fruit splitting which at the same time increases fruit size and maintain acceptable fruit quality.

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2. LITERATURE REVIEW: FRUIT SPLITTING IN CITRUS

(This section is accepted for publication in Horticultural Reviews)

2.1. INTRODUCTION

2.1.1. Problem and overview

Fruit splitting is a major pre-harvest physiological disorder in various citrus species, leading to annual yield losses of up to 30% (Barry and Bower, 1997; Rabe et al., 1990). Fruit splitting is caused by pressure resulting from the expanding pulp of an individual fruit on the rind (Almela et al., 1994; Barry and Bower, 1997; Bower et al., 1992; Erickson, 1968), eventually causing a fissure at the stylar- or navel-end, and leading to the splitting of the fruit (Fig. 2.1). Split fruit eventually drop from the tree. Not only does fruit splitting negatively affect yield, but it also attracts insects and pathogens which causes decay and require intense labour to sanitise the orchards.

A wide variety of cultural and environmental factors, independent of clonal characteristics influence and contribute to both the initiation and severity of citrus fruit splitting. These include nutrient imbalances (Bar-Akiva, 1975; De Cicco et al., 1988; Erickson, 1957), warm and humid climatic conditions (Almela et al., 1994; Barry and Bower, 1997; Coit, 1915), irregular water supply (De Cicco et al., 1988; Goldschmidt et al., 1992; Wager, 1939) and heavy crop load (Barry and Bower, 1997; Rabe et al., 1990). Secondary to these factors, fruit growth and morphological features of the fruit such as the thickness of the rind and large navels, also play an important contributing role in the initiation of the disorder (Lima et al.,

1980; Wager, 1939). The severity of the disorder may vary considerably between seasons (Almela et al., 1994; De Cicco et al., 1988), making it difficult to predict and control.

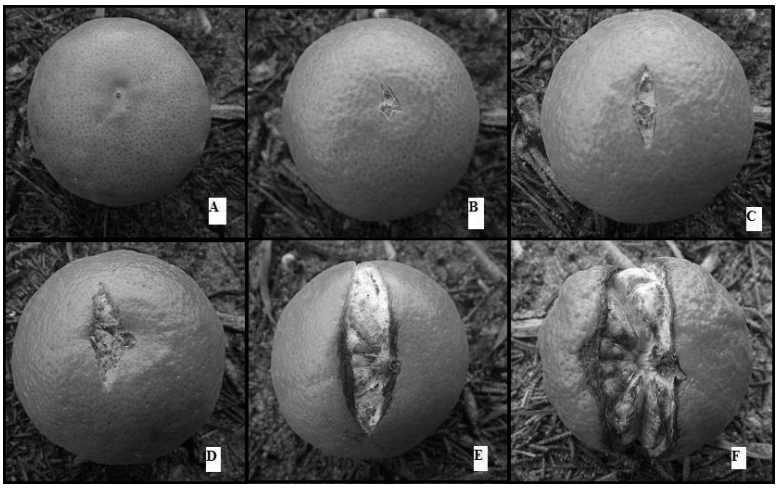

Fig. 2.1. Fruit splitting initiates at the stylar-end of the fruit as a small fissure of the rind (A). As the pulp starts to expand, the accompanied increase in volume forces the rind to split open (B-E). Split fruit eventually drop from the tree (F). Labour is required to remove split fruit from trees and abscised split fruit from the orchard floor (sanitation).

Most of the studies on the control of fruit splitting focussed on increasing the rind thickness and -strength of split-prone species by applying pre-harvest mineral nutrient sprays to the canopy or plant growth regulator (PGR) foliar sprays. Although results were erratic, PGR applications were generally more successful than mineral nutrient applications for reducing spitting. In this regard, the synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D) (Almela et al., 1994; Borroto et al., 1981; Greenberg et al., 2006; Mupambi, 2010), and gibberellic acid (GA_3) (Almela et al., 1994; García-Luis et al., 1994; Rabe et al., 1990) were most successful. Calcium (Ca) (Almela et al., 1994; Barry and Bower, 1997; Sdoodee and Chiarawipa, 2005) and potassium (K) (Bar-Akiva, 1975; Borroto et al., 1981; Greenberg et al., 2006) also reduced splitting, but to a lesser degree. Goldschmidt et al. (1992) focussed on

the control of pulp expansion during periods of split initiation, using irrigation management and achieved a significant reduction in the incidence of split fruit.

2.1.2. Cultivars particularly susceptible to split

Fruit splitting occurs worldwide as a pre-harvest problem in mandarin and mandarin hybrids (Almela et al., 1994; Barry and Bower, 1997; Goldschmidt et al., 1992) as well as 'Navel' (De Cicco et al., 1988) and 'Valencia' orange (Bower et al., 1992) (Table 2.1). Cultivars with genetically thin rinds, especially mandarin and mandarin hybrids (easy-peelers), are potentially more susceptible to split than cultivars with thicker rinds. The earliest mandarin species prone to splitting, although at low severity, is 'Owari' satsuma. In Clementine, 'Fino', 'Marisol' (Mupambi, 2010) and 'Nules' are cultivars known to be susceptible to severe levels of split fruit, while splitting has been reported in mandarin hybrids of 'Ellendale' (Rabe et al., 1989), 'Murcott' (Goldschmidt et al., 1992), 'Nova' (Almela et al., 1994) and 'Shogun' (Sdoodee and Chiarawipa, 2005) especially in years of heavy fruit load (Rabe and Van Rensburg, 1996).

'Navel' orange and certain other mandarin cultivars such as 'Nova' and 'Ellendale', genetically develop a secondary fruitlet (navel) covered or protruding at the stylar-end of the primary fruit (García-Luis et al., 1994; Rabe and Van Rensburg, 1996). The presence of these structures hamper the structural integrity of the fruit rind (García-Luis et al., 1994) and fruit with large secondary fruit (navels) are thus more prone to splitting, as was found with 'Washington navel' orange (García-Luis et al., 1994; Lima et al., 1980; Wager, 1939). In addition to the secondary fruitlet, certain cultivars such as 'Navelina' orange and 'Nova' mandarin are more split-prone due to their particular oblate shape and the high rate of

morphological differentiation from globose to oblate during critical growth periods (De Cicco et al., 1988; García-Luis et al., 2001). 'Valencia' orange is the latest maturing orange variety and generally develops fruit with thin rind and increased susceptibility to split. Cultivars of note include 'Campbell', 'Frost', 'Midknight' and 'Olinda' Valencia (Borroto et al., 1981; Bower et al., 1992).

The wide variety of influencing factors as well as the complexity of their interactions makes it almost impossible to provide a single commercial solution to the disorder. Therefore, an understanding of the physiology of both the tree and of the most important structural components of the fruit in relation to fruit splitting, *viz*., the rind and the pulp, is required to combat fruit splitting in citrus.

Table 2.1: Citrus cultivars particularly susceptible to fruit splitting.

Species	Type	Cultivar	Time of maturity	Prevalence
Mandarin	Satsuma	Owari	Early	Low
	Clementine	Fino, Marisol, Nules, Orogrande	Middle	High
	Hybrid	Murcott, Nova, Mor, Orri, Ellendale, Orlando, Ortanique, Shogun	Middle/Late	High
Orange	Navel	Hamlin, Navelina, Rustenberg, Washington	Middle/Late	High
	Valencia	Campbell, Frost, Leng Midknight, Olinda,	Late	Moderate

2.1.3. Fruit splitting in other horticultural crops

Fruit splitting or cracking has been reported for almost every horticultural crop of economic importance. This disorder occurs most notably in apple (Visai et al., 1989), apricot (Benson,

1994), cherry (Belmans and Keulemans, 1996), grape (Considine and Kriedmann, 1972), nectarine (Gibert et al., 2007), prune (Milad and Shackel, 1992) and tomato (Peet, 1992) (Table 2). Although similarities in certain causal factors as well as certain aspects of the physiological development of the phenomenon exist between these crops and citrus fruit, unique anatomical features of citrus fruit as well as the physiology of fruit development separates the phenomenon in citrus from other crops.

Splitting of a fruit is a consequence of cracks developing in the epidermis or in the rind of a developing fruit. In most crops that are prone to cracking or splitting the spatial distribution of cracks varies. Cracks could develop at the stylar- and/or stem-end, cheeks and shoulders of the fruit. Although tendency to crack is genetically controlled with certain cultivars being crack-resistant (Belmans and Keulemans, 1996; Gülşen et al., 1995; Sperry et al., 1996), the intensity of the disorder in crack-prone cultivars is episodic, with fluctuations in severity between seasons. This indicates the disorder in other crops to also be induced by either environmental conditions and/or cultural factors (Peet, 1992).

Certain causative factors in other fruit crops, *viz.* cultural and environmental, are also important with citrus. Heavy rainfall during the period of rapid fruit growth in apricot (Gülşen et al., 1995), grape (Clarke et al., 2010) as well as tomato (Peet, 1992) and at harvest in cherry (Belmans and Keulemans, 1996) correlates as a causative factor of the phenomenon in citrus fruit (Wager, 1939). In addition, as suggested in citrus (Coit, 1915), high volume irrigation after a period of water stress has been shown to result in the development of cracks in prune (Milad and Shackel, 1992), tomato (Peet, 1992) and nectarine (Gibert et al., 2007).

Table 2.2: Summary of factors causing fruit cracking and splitting in commercially important horticultural crops, as well as proposed control measures.

Crop	Cause	Control
Apple	Serious peel russet (Visai et al. 1989)	Foliar GA_{4+7} applications (Byers et al. 1990)
Apricot	B deficiency (Benson 1994) Heavy rainfall during fruit growth (Gülşen et al. 1995)	B application (Benson 1994)
Cherry	Excessive water supply at harvest (Belmans and Keulemans 1996)	Ca applications (Meheriuk et al. 1991) GA applications (Cline and Trought 2007)
Grape	High rainfall at onset of ripening (Clarke et al. 2010)	Consistent water supply (Considine and Kriedmann 1972)
Nectarine	Low crop load (Gibert et al. 2007) Increased fruit size (Gibert et al. 2007)	Lowering irrigation during rapid fruit growth (Gibert et al. 2007)
Prune	Excessive irrigation following water stress (Milad and Shackel 1992)	Consistent water supply (Milad and Shackel 1992)
Tomato	Excessive irrigation following water stress (Peet 1992); Insufficient nutrition (Huang and Snapp 2004); Low crop load (increased growth rate) (Peet 1992); Harvest at pink stage (late) (Peet 1992)	Use of crack-resistant cultivars (Sperry et al. 1996); Consistent water supply (Sperry et al. 1996); GA (Peet 1992) and Ca (Huang and Snapp 2004) application; Harvest at green-mature stage (early) (Peet 1992)

Cultural practices encouraging excessive fruit growth, such as high irrigation during the period of rapid fruit growth of nectarine (Gibert et al., 2007) as well as pruning and thinning of tomato plants in a similar developmental phase (Peet, 1992) can lead to the development of cracks of the fruit surface. In terms of mineral nutrient deficiency, cracks develop due to

boron (B) deficiency in apricots (Benson, 1994) and calcium (Ca) deficiency in cherry (Meheriuk et al., 1991) and tomato (Huang and Snapp, 2004).

Even though similarity exists in causes of splitting between citrus and other fruit the unique morphology and physiology of citrus fruit indicate additional causes. Citrus fruit is known as a Hesperidian berry, with a leathery rind and internal swollen trichomes and juice sacs (Swingle, 1943), these aspects as well as the differences in rind thickness and presence or absence of secondary fruit, add factors that could cause the initiation and splitting of citrus fruit.

2.2. PHYSIOLOGY OF CITRUS FRUIT SPLITTING

2.2.1. Relationship between fruit growth, resulting -shape and splitting

Citrus fruit growth follows a sigmoïdal curve as described by Bain (1958), with the rind developing mainly during stage I and II, whereas pulp growth predominantly occurs during stage II. During stage III, very little increase in fruit size occurs as the fruit matures.

García-Luis et al. (2001) linked the chronological development of this disorder with certain morphological changes of the fruit during development, such as an increase in diameter/height-ratio. The physical splitting of the fruit predominantly occurs during stage II of fruit development of split-prone cultivars (Fig. 2.2) (Borroto et al., 1981; García-Luis et al., 1994; Goldschmidt et al., 1992; Rabe and Van Rensburg, 1996). During stage II of fruit development there is an increase in pulp volume, due to the expansion of cells caused by increase in turgor pressure, resulting in a progressive change in fruit shape.

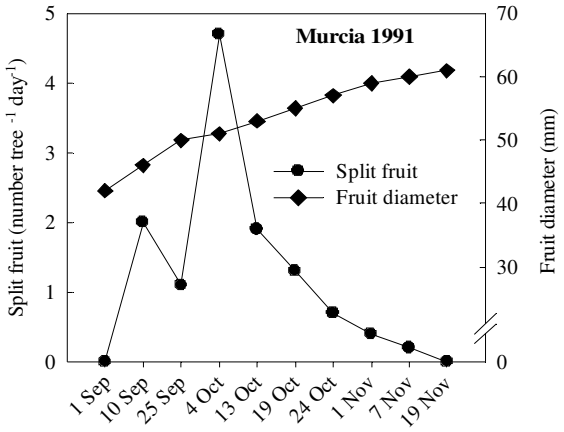

Fig. 2.2. The seasonal pattern of fruit splitting, expressed as number of split fruit per tree per day, as it occurred in 'Nova' mandarin in an orchard in Spain (Murcia) (Northern hemisphere) in 1991. The initiation of splitting corresponds with the rapid increase in fruit diameter [Adapted from García-Luis et al., (1994)].

Fruit shape is classified as the ratio between fruit diameter and fruit height. Fruit with a low diameter to height-ratio (D/H-ratio) are generally referred to as globose. During the period of maximum increase in citrus fruit diameter (stage II), fruit transform from globose to oblate in shape (García-Luis et al., 2001) and pressure exerted by the pulp forces the rind to enclose its expanding volume due to the predominant occurrence of the pulp and albedo's cell growth (Bain, 1958). The increase in fruit D/H-ratio is accompanied by an increase of internal stress exerted on the poles of the fruit (Considine and Brown, 1981), namely the calyx- and the stylar-end.

While the large intercellular spaces of the albedo absorb some of the pressure exerted by the rapid pulp expansion (Monselise, 1986), the flavedo stretches and becomes thinner. The flavedo of split-prone cultivars cannot accommodate the increase in pulp volume (García-

Luis et al., 2001; Goldschmidt et al., 1992; Erickson, 1957) and as a result, fruit split at the stylar- or navel-end, where the rind is thinner and structurally weaker (Coit, 1915).

Although fruit shape is specific to a cultivar, certain factors such as warm temperature, relative humidity as well as rapid water uptake by the tree (Goldschmidt et al., 1992; Lima et al., 1980; Wager, 1939) may accelerate fruit growth. This could alter the fruit shape (increase the D/H-ratio) to such an extent that the rind is unable to accommodate the increasing pulp volume and the fruit splits (Goldschmidt et al., 1992). In 'Navel' orange, as the D/H-ratio is reduced, a lower incidence of fruit splitting occurred (De Cicco et al., 1988). In 'Nova mandarin', the percentage split fruit reached a maximum when the D/H-ratio increased from 1.21 to 1.23 during fruit growth (Fig. 2.3) (García-Luis et al., 2001).

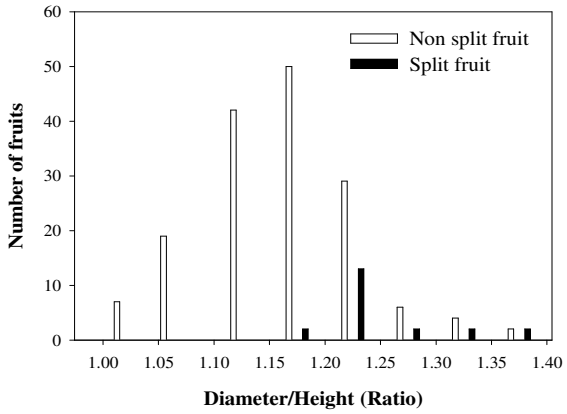

Fig. 2.3. Altogether 200 fruit from 'Nova mandarin' trees were tagged and their diameter and height periodically measured until the end of fruit development at which they were then classed as split (solid bars) and non-split (hollow bars). Final diameter/height ratio of all tagged fruit ranged from 1.02 – 1.37 with split occurrence at ratios ≥ 1.20 [Adapted from García-Luis et al., (2001)].

As reported for other crack-prone crops such as tomato (Peet, 1992) and nectarine (Gibert et al., 2007), a reduction in irrigation rate during periods of rapid fruit growth, without stressing the tree, holds promise in reducing the likelihood of fruit cracking and splitting. By reducing water supply during stage II of fruit development, fruit splitting was reduced in 'Murcott' mandarin (Goldschmidt et al., 1992).

Although the physical splitting of the citrus fruit occurs as a consequence of increase in pulp volume during stage II of fruit development, the potential for fruit splitting incidence is to a large extent a result of any stress to the tree and young fruitlets during fruit development in stage I (Rabe and Van Rensburg, 1996). Factors such as nutritional imbalances, water stress during stage I, high flower number and fruit set hamper sufficient cell division and lead to the development of a weak and thin rind. In addition, fruit shape (known to be genetically influenced) and ambient growing conditions, affect the initiation and severity of fruit splitting of the rind. Therefore, finding a successful commercial solution to fruit spitting of citrus will require an understanding of how to manipulate not only fruit growth, but in particular fruit rind development.

2.2.2. Splitting as related to rind characteristics

Although fruit with high D/H-ratios are more likely to split, a proportion of fruit from the same tree with similar dimensions or even higher, remains structurally intact. Therefore it could be assumed that additional aspects in the fruit rind development could increase its susceptibility to splitting in reaction to expanding pulp. Cultural as well as environmental factors contribute to the development of citrus fruit with thin or weak rind and indirectly, the fruit's propensity to split (Fig. 2.4) (Almela et al., 1994). As cell division in the fruit rind

predominantly occurs during stage I of fruit development, contribution of these factors to increased susceptibility to splitting predominantly occurs during stage I of fruit development (Rabe and Van Rensburg, 1996).

Cultural factors include imbalances of certain mineral nutrients such as K and P (Bar-Akiva, 1975; Chapman, 1968; Morgan et al., 2005), hormonal imbalances due to production of seedless fruit (Erner et al., 1976; Rabe et al., 1990) and suboptimal irrigation, hampering plant-soil water relations. Any alterations leading to increased inter-fruit competition in the tree, such as girdling or GA_3 treatments that stimulate a higher percentage fruit set, could lead to the development of fruit with a thin or weak rind and an increase in splitting (Rabe et al., 1990). The most important environmental factors leading to development of fruit with thin rind and thus also susceptibility to splitting, are warm and humid climatic conditions during stage I of fruit development (Cohen et al., 1972).

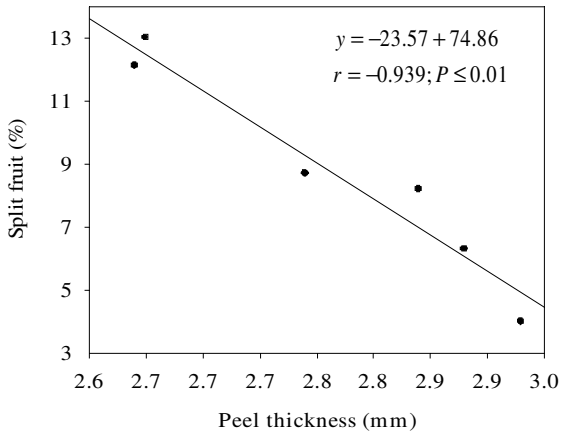

Fig. 2.4. As rind thickness of 'Nova mandarin' hybrid increases, the percentage split fruit decreases, indicating an inverse relationship between fruit splitting and rind thickness in 'Nova mandarin' fruit [Adapted from Almela et al., (1994)].

2.3. CAUSES OF CITRUS FRUIT SPLITTING

2.3.1. Cultural

Mineral nutrition

With regard to citrus fruit splitting, imbalances in potassium (K) and phosphorous (P), can contribute to thin or weak rind and can therefore, at either too high or low rates, indirectly increase the likelihood of splitting (Almela et al., 1994; Bar-Akiva, 1975; Borroto et al., 1981; Morgan et al., 2005).

With an increase in P-supply and hence increased leaf P-content, a decrease in rind thickness of citrus fruit is found (Chapman, 1968). In contrast, an increase in leaf K-content results in an increase in rind thickness and coarseness (Chapman, 1968; Monselise, 1986). This concurs with results found on the mineral composition of split 'Washington' navel oranges, with rind of split fruit having significantly higher P-content than non-split fruit (Erickson, 1957). Similarly, a decrease in rind thickness of split fruit was found as the leaf P-content increased in 'Navelina' orange and a reduced number of split fruit as the leaf K-content increased (De Cicco et al., 1988). Higher incidence of splitting in trees with low leaf K-content was reported in 'Hamlin' (Koo, 1961) and 'Valencia' orange (Gilfillan and Stevenson, 1982). Morgan et al. (2005), reporting an increase in rind thickness of 'Hamlin' orange with an increase in K nutrition and without evaluating the effect on number of split fruit, hypothesized that an increase in rind thickness could lead to a reduction in fruit splitting of this split-prone cultivar.

One of the aims with fertilizer practices is to ensure an optimum for both P and K in citrus orchards. Optimum for leaf P ranges between 0.10% and 0.16% and for leaf K, between 1.0% and 1.5% (CRI production guidelines, 2007). Some orchards with these nutrient levels in leaves still have a high percentage split fruit. This indicates the involvement of factors other than P and K contributing to the prevalence of this disorder.

Hormonal imbalances

Growth and development of citrus fruit is to a great extent dependent on the endogenous hormone content of the fruit. Seeds that develop as a result of pollination, serve as a source of these hormones during the critical early development stage of the fruit (Monselise, 1977).

However, split-prone mandarin cultivars such as 'Nova' and 'Ellendale' are weakly parthenocarpic and planted in isolated blocks to avoid cross-pollination and resultant seed formation (Barry and Bower, 1997). Production of seedless fruit cannot only lead to reduced fruit set, but the lack of seeds has been correlated with a decrease in rind thickness and a resultant higher occurrence of fruit splitting. Cross-pollinated 'Ellendale' mandarin trees had more seeds than non-cross-pollinated trees and thus also an increased rind thickness and very few split fruit (Rabe et al., 1990). Pollination and seed formation increases the gibberellin content of weakly parthenocarpic fruit (Ben-Cheikh et al., 1997). It is possible that this increased endogenous gibberellin content leads to an increase in rind thickness. High levels of endogenous GA_3 and cytokinin are responsible for the excessive growth of 'Shamouti' orange fruit rind, which leads to the development of a very thick and rough rind (Erner et al., 1976).

Rainfall and irrigation

Splitting of citrus fruit is a consequence of the pressure of expanding pulp on the rind of citrus fruit (García-Luis et al., 1994; García-Luis et al., 2001; Goldschmidt et al., 1992). It could therefore be reasoned that any factor leading to drastic or excessive pulp expansion, could increase the incidence and severity of splitting. The influx of water into the pulp during stage II and III of fruit development could exert undue pressure on the developing rind and lead to the eventual splitting thereof (Coit, 1915; Goldschmidt et al., 1992; Lima et al., 1980; Wager, 1939).

Avoiding fluctuations in soil water content, as well as avoiding depletion of water from deeper soil layers is of critical importance in avoiding fruit splitting. This was illustrated by De Cicco et al. (1988) who reported a significantly higher severity in fruit splitting of 'Navelina' orange when the total available water in the 40-80 cm soil layer was low. With total available water in the 40-80 cm soil layer at an optimum, fruit splitting was significantly lower. In reaction to daytime drought stress, citrus leaves can withdraw water from the rind (xylem backflow), which leads to cessation of, or decrease in fruitlet growth (Furr and Taylor, 1939; Hilgeman, 1977). This is thought to result in a premature strengthening of the flavedo cells, leading to a reduced ability to divide and enlarge as normal (Graebner, 1920). Wager (1939), suggested the initiation of splitting of 'Washington Navel' orange fruit to be caused by a sudden supply of large amounts of water after a period of water stress, during which the prematurely strengthened cells are unable to react to the sudden re-supply of water into the pulp. Large tension exerted on the rind leads to eventual failure of the rind tissue. A similar mechanism was proposed to be responsible for splitting of tomato (Peet, 1992) and prune (Milad and Shackel, 1992).

Citrus produced in the humid, summer rainfall areas of South Africa have a higher propensity to split, presumably due to the frequent and unavoidable natural water supply during periods of rapid fruit growth. However, Rabe and Van Rensburg (1996) failed to correlate the seasonal rainfall pattern positively with fruit splitting.

Crop load

The severity of citrus fruit splitting is very much dependent on flower number, percentage fruit set (Rabe et al., 1989; Rabe and Van Rensburg, 1996) as well as final crop load (Barry and Bower, 1997; Gilfillan and Stevenson et al., 1984; Rabe et al., 1990). A high percentage of split fruit occurred at very high crop loads and little or no splitting in years of low crop loads (Gilfillan and Stevenson et al., 1984). However, an increase in the disorder was expected at lower crop loads in tomato (Peet, 1992) and nectarine (Gibert et al., 2007), which can be explained by the higher growth rate of individual fruit at lower crop loads and an inability of the fruit epidermis to accommodate for the increase in fruit volume created by these higher growth rates.

With an increase in the number of fruit per tree, a linear increase in the inter-fruit competition for water and assimilates is expected. Lenz and Cary (1969) reported a decrease in fruit size and more importantly, a decrease in rind thickness of citrus fruit with an increased crop load per tree. As crop load increased, the K concentration, a very important contributing agent to the development of a healthy and thick rind, declined in both the leaves and shoots of citrus trees (Lenz, 2000).

Cultural practices such as girdling or GA_3 treatments during the blossom period are generally applied to increase fruit set percentage in citrus, especially in weakly parthenocarpic cultivars

(Barry and Bower, 1997; Rabe et al., 1990; Rabe and Van Rensburg, 1996). However, the higher set percentage results in an increased inter-fruit competition, leading to a significantly higher number of split 'Nova' and 'Ellendale' mandarin fruit (Barry and Bower, 1997; Rabe and Van Rensburg, 1996).

Canopy position microclimate

Growth habit and resulting tree shape of some citrus cultivars as well as ineffective pruning by producers, stimulates the development of variation in micro-climate within a tree canopy that could lead to the development of fruit that are potentially more prone to physiological disorders such as rind breakdown of 'Nules Clementine' mandarin (Cronjé et al., 2011).

However, the fruit in the outer canopy are exposed to higher temperatures than inside fruit. The high surface temperature of fruit in the outer canopy can cause the formation of free radicals inside cells of the fruit and their high reactivity leads to a loss in membrane integrity and eventual cell death (Wünsche et al., 2004). In 'Stayman' apple, fruit in the outer canopy are more likely to crack (Verner, 1935). In citrus the cellular damage due to the high heat leads to sunburn in the flavedo (light yellow to brown discolouration) and has been described by Sdoodee and Chiarawipa (2005) as a causal factor of splitting of 'Shogun' mandarin in Thailand.

2.3.2. Environmental

Climate has a definitive effect on rind thickness as well as the rate of fruit growth, thereby indirectly affecting the propensity of fruit toward splitting. Almela et al. (1994) reported

varying intensities of 'Nova' mandarin fruit splitting between years and linked the variability to seasonal difference in climate.

Temperature

Barry and Bower (1997) reported the splitting of 'Nova' mandarin fruit to be less prevalent in cooler production regions. This was in agreement with Coit (1915), who suggested that regions more prone to hot weather are more likely to experience fruit splitting. Reuther et al. (1973) found that 'Valencia' orange exposed to warmer climate during the rapid growth period, developed thinner rind and thus experienced higher levels of fruit splitting, compared to fruit exposed to lower temperatures. In these hot areas, the growth rate of citrus fruit during every stage of fruit development is also higher than those in cooler areas. This accelerated growth rate, especially of the pulp during growth stage II of fruit development, may lead to higher pressure being applied to the rind and thus initiate fruit splitting. This is similar to what Peet (1992) found in tomato, when fruit are exposed to high temperatures.

Humidity

Citrus fruit grown in humid production regions develop thinner rinds than those grown in drier regions (Cooper et al., 1963) and are therefore, more likely to split. Rabe et al. (1989) reported severe fruit splitting of 'Ellendale' mandarin specifically in hot and humid citrus producing regions in Swaziland (Tambankulu) and South Africa (Letaba, Limpopo), compared to regions with lower humidity such as the Western Cape Province of South Africa. However, a high incidence of fruit splitting is often associated with a dry spring followed by a wet period during stage II and III of fruit development.

2.4. REDUCING CITRUS FRUIT SPLITTING

2.4.1. Foliar mineral nutrient applications

Potassium

The mineral nutrient most abundant in a citrus fruit is K, and in K-deficient trees, reduction in fruit size as well as number of fruit is expected (CRI production guidelines, 2007). The most important effect of increased K-nutrition on quality of citrus fruit is a reduction in the juice content and total soluble solids (TSS) as well as an increase in fruit size, total acidity (TA) and rind thickness. Therefore, supplementing split-prone cultivars with K to achieve leaf K-levels of between 1.00 % and 1.50 % (CRI production guidelines, 2007), will lead to the development of fruit with thicker rind that are less likely to split (Morgan et al., 2005).

The soil application of K on three-year old container-grown 'Valencia' orange trees reduced fruit splitting and increased rind thickness of K-treated trees, compared to untreated trees that experienced a higher incidence of fruit splitting (Bar-Akiva, 1975). Potassium is readily translocated within a citrus tree and soil fertilization is the most effective cultural practise to address nutrient deficiency in citrus trees. Foliar sprays do, however, result in some nutrient uptake and could be used as a method of supplementing trees with K or correcting any deficiency of K. The time of foliar K-application affects its efficacy in terms of influencing the incidence of splitting and Rabe et al. (1989) reported higher numbers of split fruit per tree when 4% KNO_3 was applied prior to physiological fruit drop. In contrast, later application resulted in a significant reduction in splitting. The higher fruit set and increase in inter-fruit competition as a result from the earlier K-application, was thought to have led to the higher splitting (Rabe et al., 1990).

Calcium

Calcium is one of the most important structural components of cell membranes and walls and plays an important role in the processes of cell division and growth (Hepler, 2005). Transport of Ca from the soil to developing fruit is a passive process, dependent on the flow of the transpiration stream through the plant. Ca is weakly translocated from old leaves or other plant parts/organs to newly developing leaves, meristems and fruit (Hanger, 1979). Therefore, any deficiency of Ca in the soil, or any factor hampering the transport of Ca, (e.g. low VPD) especially during critical periods of cell division and growth, will lead to Ca-deficiency in the fruit. Such a deficiency or imbalance could lead to physiological disorders involving the rind *viz*., rind breakdown of 'Nules Clementine' mandarin (Cronjé et al., 2011) and fruit splitting of 'Shogun' mandarin (Sdoodee and Chiarawipa, 2005). The optimum leaf Ca in citrus production is 2.5 - 5.5 % (CRI production guidelines, 2007).

Cracking of tomato (Huang and Snapp, 2004; Peet, 1992) and cherry (Meheriuk et al., 1991) is thought to be a consequence of insufficient Ca-supply and multiple foliar applications of $CaCl_2$ and $Ca(OH_2)$ reduced the incidence of cracking in 'Van' cherry. This concurred with an earlier study by Barry and Bower (1997), in which a single foliar application of 2% $Ca(NO_3)_2$ at 70% full bloom significantly reduced fruit splitting in 'Nova' mandarin, compared to control, untreated trees. Boron (B) and Ca interact to form a stabilizing complex in the middle lamella of plant cells (Blevins and Lukaszewski, 1998). By applying these two mineral nutrients in combination as a foliar spray to crack-prone 'Mountain spring' tomato, there was a significant reduction in the number of cracked tomato fruit (Huang and Snapp, 2004). A similar significant reduction of split 'Shogun' mandarin fruit was recorded after the foliar application of either 1% $CaCl_2$, boric acid or a combination thereof, was applied 4 months after fruit set (Sdoodee and Chiarawipa, 2005).

2.4.2. Foliar-applied plant growth regulator (PGR) applications

Foliar applications of PGRs result in varying amounts of success in reducing fruit splitting of split-prone cultivars. PGRs could strengthen the rind of developing fruitlets (Coggins and Hield, 1968) or inhibit rind senescence (García-Luis et al., 1994). Application of PGRs in the form of auxin and gibberellin could potentially substitute for the lack of natural endogenous hormones in seedless cultivars and increase the rind strength of these fruit and their resistance to split.

In a study on the effect of applied hormones on the anatomy and splitting of 'Nova' mandarin, García-Luis et al. (1994) observed a decrease in fruit splitting attributed to applied GA_3 successfully inhibiting the senescence of the rind, making the fruit less prone to splitting. The application of the synthetic auxin 2,4-D to cultivars prone to develop thin and/or smooth rinds, led to an increase in thickness and rind coarseness (Coggins and Hield, 1968) which has subsequently been shown to reduce fruit splitting (García-Luis et al., 2001).

However, in some instances PGR treatments resulted in contrasting results and either promoted fruit splitting in 'Navel' orange (Lima and Davies, 1984), had little or no effect on 'Valencia' orange (Gilfillan and Stevenson, 1982) or reduced the disorder on a number of mandarin species (Almela et al., 1994; Borroto et al., 1981; García-Luis et al., 1994; García-Luis et al., 2001; Greenberg et al., 2006; Mupambi, 2010).

Gibberellic acid (GA_3)

Gibberellins are plant hormones responsible for facilitating cell division and enlargement and therefore, the application of GA_3 is used in many crops, such as grape and cherry to increase

fruit size (Cline and Trought, 2007). Apart from its effect on fruit size, GA_3 applications also increase firmness of crack-prone cherry (Cline and Trought, 2007) and tomato fruit (Peet, 1992). In citrus, GA_3 increases rind resistance to pressure and delay of chlorophyll breakdown (McDonald et al., 1987), which is an indication of delaying of rind senescence (García-Luis et al., 1994) and its potential as a possible control measure of fruit splitting in certain citrus species. In 'Washington Navel' orange, Bevington (1973) reported an increase in rind resistance to puncturing as well as a decrease in physiological disorders of the rind, such as puffing and creasing, with May (Southern hemisphere) applications of GA_3.

In 'Nova' mandarin, GA_3 (20 $mg \cdot L^{-1}$) applied after physiological fruit drop was more successful than a foliar spray during full bloom, in reducing fruit splitting (García-Luis et al., 1994; García-Luis et al., 2001). This corresponds with the increase in fruit splitting observed when GA_3 applications at 10 or 20 $mg \cdot L^{-1}$ were made during full bloom on 'Ellendale' mandarin (Rabe and Van Rensburg, 1996). This can be explained by an increase in fruit set from the earlier application and therefore, inter fruit competition.

Synthetic auxin: 2,4-dichlorophenoxy acetic acid (2,4-D)

Expansion of plant cells is facilitated by the important plant hormone auxin, produced in apical meristems and transported to growing tissue (Coggins and Hield, 1968). Commercial applications of synthetic auxins in citriculture include 2,4-dichlorophenoxy propionic acid (El-Otmani et al., 1993) and 3,5,6-trichloro-2-piridil oxyacetic acid (Greenberg et al., 2006) and are applied to increase fruit size by facilitating increased cell expansion (Mitchell, 1961).

Excessive stimulation of cell expansion can lead to eventual cell and plant death, as is the case with application of the synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D) as a

lethal herbicide. Some crops, such as grape are very sensitive to this particular PGR and drifts of herbicidal sprays often lead to death of vines (Kasimatis et al., 1968). However, citrus trees are less sensitive to this PGR, and when applied at low concentrations (≤20 mg·L^{-1}), an increase in fruit size is expected due to increased sink strength of treated fruit as well as stimulation of rind growth (Guardiola and García-Luis, 1997).

In preliminary studies to reduce the size of navel-end opening in 'Navel' orange, a significant decrease in fruit splitting of 'Marisol Clementine' by foliar application of 2,4-D at full bloom (25 mg·L^{-1}) and petal drop (15 mg·L^{-1} and 25 mg·L^{-1}) occurred (Mupambi, 2010). These treatments, although successfully reducing fruit splitting, negatively increased rind coarseness and led to an increased percentage fruit with persistent styles. Application of 2,4-D on the same cultivar and site, but at later timing (after physiological fruit drop) and at 15 mg·L^{-1} reduced fruit splitting and produced fruit of acceptable rind coarseness and without persisting styles, indicating success of this treatment could be dependent of timing as well as concentration (unpublished). García-Luis et al. (2001) observed a significant reduction of fruit splitting with foliar applications of 20 mg·L^{-1} 2,4-D at full bloom, and attributed it to an increase in rind thickness at the stylar-ends of treated fruit.

Combination of PGRs and mineral nutrients as foliar treatments

The combination of PGRs and mineral nutrients proved successful in reducing the severity of citrus fruit splitting. The disorder was reduced by more than 50%, with a treatment consisting of KCl (150 kg·ha) and 2,4-D at 10 mg·L^{-1} on 'Olinda Valencia' orange trees (Borroto et al., 1981). Greenberg et al. (2006) found a reduction of fruit splitting when 5% Bonus-NPK was applied with 2,4-D foliar sprays on 'Nova' mandarin. Almela et al. (1994) reported a foliar

spray of 2% $Ca(NO_3)_2$, 20 $mg \cdot L^{-1}$ 2,4-D and 20 $mg \cdot L^{-1}$ GA_3 reduced fruit splitting by increasing rind strength, but not rind thickness, which could indicate a possible strengthening effect of the citrus fruit rind.

2.4.3. Managing plant water relations

Careful and detailed management of irrigation practices during fruit growth periods, with the aim of avoiding fluctuations in soil water content, is key in controlling fruit splitting in citrus (Rabe, 1988). Goldschmidt et al. (1992) achieved moderate success in reducing splitting of 'Murcott' mandarin when they reduced the normal irrigation during late summer in the period of pulp expansion (stage II) to 50-60% and as much as 30% of normal irrigation, without significantly affecting internal fruit quality. Although deficit irrigation during late stages of fruit development is a standard practice to increase the total soluble solid content (TSS) of early maturing cultivars such as 'Satsuma' mandarin, it is important not to stress trees excessively during the early stages of fruit development, as it may negatively affect fruit growth and final fruit size (Hilgeman, 1977).

2.4.4. Thinning

Splitting of citrus has been particularly prevalent in years of excessive crop load (Rabe and Van Rensburg, 1996), where high competition for water and assimilates between fruit lead to a reduction in total partitioning between fruit and the development of small, thin-rinded and split-prone fruit. Therefore, thinning of flowers or thinning of fruit prior to physiological fruit drop (stage I) in years of expected, excessive crop load is suggested as a possible measure to

reduce competition between fruitlets for assimilates and water. This can increase partitioning to individual fruit to eventually produce larger fruit with thicker and stronger rind, and an accompanied lower incidence of splitting (Barry and Bower, 1997). In addition, in years where a heavy crop load is expected, both irrigation and nutrition should be adjusted to accommodate for the increased fruit set.

However, excessive thinning of fruitlets or thinning during the period of rapid fruit growth (stage II), may result in a low fruit set percentage and as a result, increase the growth rate of remaining fruit to such an extent that the rind of these fruit are unable to accommodate for the increased growth rate and result in fruit cracking or splitting. The thinning of young fruit was proposed as a causative factor in the cracking of nectarine (Gibert et al., 2007) and tomato fruit (Peet, 1992) due to the resulting increase in growth rate of remaining fruit.

2.5. CONCLUSION

Fruit splitting in citrus differs from other crops due to the unique morphology of citrus fruit, consisting of the pulp and rind which is in addition made up of the spongy white internal layer, the albedo (mesocarp) and an external layer, the flavedo (exocarp) (Monselise, 1986).

During stage I of fruit development (cell division), the majority of the flavedo cells are formed where after cell division of the flavedo declines and cells of the pulp start to expand in stage II (Bain, 1958). The pressure applied by the rapidly expanding pulp leads to the formation of micro-cracks and initiation of splitting at the stylar- or navel-end of the fruit, the area where the rind is the thinnest and/or structurally weaker than other areas of the rind.

Certain environmental as well as cultural factors lead to the development of rind that is thinner and more split-prone. Such factors include nutrient imbalances, specifically low K and P (Bar-Akiva, 1975; De Cicco et al., 1988; Erickson, 1957), warm and humid climatic conditions (Almela et al., 1994; Barry and Bower, 1997; Coit, 1915), irregular water supply (De Cicco et al., 1988; Goldschmidt et al., 1992; Wager, 1939) and heavy crop loads (Barry and Bower, 1997; Rabe et al., 1990). However, stress during stage I of fruit development seems to be the most important factor determining the susceptibility of an orchard to fruit splitting (Rabe and Van Rensburg, 1996). During this stage, the majority of the flavedo cells are formed and its structural integrity determined. With high crop loads, or by insufficient irrigation or fertilisation during this critical period, the potential for fruit to split is increased significantly.

Fruit splitting has been reported in various 'Valencia' as well as 'Navel' orange cultivars, which are more prone due to the presence of a secondary fruitlet (navel) that weakens the structure of the primary fruit. Fruit splitting occurrence is more severe in thin-rind, reticulated mandarin and mandarin hybrids (Almela et al., 1994; Barry and Bower, 1997; Goldschmidt et al., 1992). Consumer preference for easy-peeling, low-seeded mandarins of high internal fruit quality, especially relatively new cultivars such as 'Murcott', 'Nova', 'Mor' and 'Orri', have led to large scale planting of these cultivars by producers. However, these cultivars are split-prone and large quantities of fruit are lost solely due to fruit splitting, especially in 'on'-years as these trees are prone to alternate bearing.

Current horticultural practices to reduce the incidence of splitting include crop load manipulation by thinning and GA_3-application/girdling, as well as sufficient Ca, K and P

nutrition and consistent irrigation. However, no single practice consistently reduces the incidence of fruit splitting in a splitting prone orchard or cultivar.

Recent research on mandarin species reported significant reduction of fruit splitting through the application of a synthetic auxin, 2,4-D as a foliar spray (Greenberg et al., 2006; Mupambi, 2010). Successful reduction of fruit splitting with foliar application of 2,4-D seems more likely on mandarin cultivars as results were erratic when applied to orange cultivars (Gilfillan and Stevenson, 1982; Lima and Davies, 1984). Additional negative effects on fruit quality, such as increased rind coarseness and reduction in juice content, is one of the major drawbacks of 2,4-D application. Successful implementation of 2,4-D application in citrus production in order to reduce splitting would be dependent on elucidating the timing, rate, as well as cultivar sensitivity.

LITERATURE REVIEW: STUDIES ON FRUIT SIZE IN CITRUS

2.1. INTRODUCTION

Fruit size is one of the most important parameters determining the profitability of citrus production. Markets have specific demands for fruit size and offer higher rewards for fruit of optimum size (El-Otmani et al., 1993; Guardiola & García-Luis, 2000). The production of fruit of unsatisfactory size, however, lead to the reduction in profitability with production costs in some instances higher than rewards offered by consumers (Guardiola and García-Luis, 1997; Guardiola & García-Luis, 2000; Gilfillan, 1987). Factors influencing fruit growth and eventual fruit size can be divided into two groups: those out of the producer's control,

such as climate, soil- and rootstock type and those that can be managed by the producer, such as water supply, nutrition, flower number and fruit load (Gilfillan, 1987).

Techniques used by producers to ensure optimum fruit size include girdling, pruning, optimum irrigation and fertilization. As fruit size is inversely related to flower number and eventual fruit load (Barry and Bower, 1997; Lenz and Cary, 1969), producers make use of thinning methods to reduce inter-fruit competition and increase fruit size (Guardiola, 1997). Except for hand thinning, which requires large amounts of labour, foliar applications of synthetic auxins, are particularly successful in this regard, reducing fruit load and/or increasing final fruit size (Guardiola and García-Luis, 1997).

Application of synthetic auxins at later timings (after physiological fruit drop), have a direct enhancing effect on rate of fruit growth and final fruit size and measure of success is dependent on concentration applied, fruit load and auxin type (Guardiola and García-Luis, 1997). The effect of various timings and concentrations of the synthetic auxin, 2,4 - dichlorophenoxy acetic acid (2,4-D), on fruit size of different citrus cultivars under South African conditions, are not well known. In preliminary studies to this project, an increase in fruit size of 'Marisol' Clementine with the application of the isopropyl ester of 2,4-D at 10 $mg \cdot L^{-1}$, during full bloom or petal drop occurred. In a recent study in Israel a significant fruit size increase of 'Nova' mandarin occurred with a treatment consisting of 40 $mg \cdot L^{-1}$ 2,4-D and 5% Bonus-NPK, applied at fruit diameter of 13 mm. (after physiological fruit drop) (Greenberg et al., 2006). Kassem et al. (2011) in a study on 'Washington' navel, reported a significant increase in fruit size with a treatment of 2,4-D (20 $mg \cdot L^{-1}$) in combination with calcium chloride (0.5%) applied at fruit diameter of 15 mm.

2.2. FACTORS INFLUENCING FRUIT SIZE OF CITRUS

2.2.1. Climate

The growth and development stages of 'Valencia' orange fruit are divided into three distinct stages and follow a sigmoidal pattern, which holds true for most citrus cultivars (Bain, 1958). The effect of the various aspects of climate on fruit growth and eventual size are mainly determined by the exposure of trees to these conditions during a specific stage of fruit development (Cooper et al., 1963). These climatic factors include sunlight exposure, humidity and wind, but the most important climatic factor influencing all biological processes and thus also fruit size, is temperature.

Stage I

During stage I of fruit development, cell division and as a result, the formation of the cellular structures of the fruit occur (Bain, 1958). Cooper et al. (1963) and Richardson et al. (1997) proved that both day and night temperatures during this period affect fruit growth and eventual fruit size of 'Valencia' orange and 'Satsuma' mandarin. In both instances, fruit grown in areas with warm night temperatures during spring exhibited accelerated growth rates compared to those grown in areas with cooler night temperatures (Cooper et al., 1963; Richardson et al., 1997). Susanto et al. (1991) evaluated the effect of both day and night temperatures prior to and during flowering on fruit growth and found that pummelo fruit exposed to 25°C day and between 10°C and 20°C night temperatures were significantly larger than those exposed to 20°C day temperature. This indicates that high temperature during initial development leads to an increased growth rate. However, temperatures

exceeding 30°C lead to rapid decrease in growth rate of fruit and damage of fruit at temperatures reaching and exceeding 40°C (Reuther, 1973).

Stage II

During stage II of fruit development, cell growth and expansion of the pulp occur and are responsible for most of the increase in fruit diameter and weight (Bain, 1958). During this stage fruit growth are more limited by too high temperatures (exceeding 38°C) (Hilgeman et al., 1959) rather than low temperatures.

Stage III

Very little growth takes place during stage III of fruit development (Bain, 1958) and final fruit size are less influenced by climatic conditions during this period, compared to stage I and II.

2.2.2. Type of rootstock

Although the type of rootstock has a definite effect on fruit size, the effect is not the single most important aspect and additional vital factors to consider include disease-, nematode- and drought resistance of the rootstock, as well as the scion type to be grafted and impact of the rootstock on specific fruit qualities to be enhanced.

Trees grafted on more vigorous rootstocks such as 'Rough' lemon [(*Citrus jambhiri* (Lush)], Rangpur lime [(*Citrus limonia* Osbeck)] and Volkameriana [*C. volckameriana* (Ten. and Pasq.)] generally produce fruit of bigger size (Economides and Gregoriou, 1993; Gilfillan, 1987; Hutchinson and Hearn, 1977). Hilgeman (1977) reported that 'Valencia' oranges

grafted on 'Rough' lemon are also more resistant to water stress than those on Troyer citrange (*C. sinensis* [L.] Osbeck x *P. trifoliata* [L.] Raf.), sour orange (*C. aurantium* L.), and Cleopatra mandarin (*C. reshni* Hort. Ex. Tan.). 'Rough' lemon and Volkameriana rootstock are also resistant to Citrus Tristeza Virus (Economides and Gregoriou, 1993; Gilfillan, 1987), but 'Rough' lemon is sensitive to *Phytophtora* (Gilfillan, 1987).

As growth vigour in rootstock decrease, those of intermediate level such as Troyer and Carrizo citranges (*C. sinensis* [L.] Osbeck x *P. trifoliata* [L.] Raf.) and sweet orange (*C. sinensis* [L.] Osbeck), produce fruit of intermediate size (Gilfillan, 1987; Hutchinson and Hearn, 1977). The Troyer and Carrizo rootstocks are nematode-resistant and generally produce fruit with good internal quality (Gilfillan, 1987). Rootstocks associated with small fruit size are the Cleopatra mandarin, the Pomeroy trifoliate and the Morton citrange (Gilfillan, 1987).

2.2.3. Fertilization

The most important nutrients affecting citrus fruit size is nitrogen (N) and potassium (K) (Chapman, 1968; Gilfillan, 1987). More than 40% of the total dry matter of citrus fruit consists of K. Therefore, any deficiency of this element may lead to a reduction in fruit size (Chapman, 1968). The positive effect of K fertilization on fruit size is well documented and increased production from K fertilization and with leaf K at an optimum range of between 1.5% and 1.7%, is expected (Koo, 1961). Morgan et al. (2005) reported an increased fruit diameter of 'Hamlin' oranges with leaf K content between 1.2% and 1.7%. K-content in the leaves are considered as low and insufficient at or below a concentration of 0.7% (Morgan et al., 2005). Page et al. (1963) reported an increase in fruit size with K application especially

when leaf content was low. This was supported by Bar-Akiva (1975), who found a significant increase in the K-content of the leaves from 0.69% to 1.36% after a K application and resulting increase in fruit size of three-year-old, container-grown 'Valencia' orange trees. However, excessive K-fertilization may lead to an increased rind coarseness and thickness (Chapman, 1968; Morgan et al., 2005).

Nitrogen (N) is also important for increasing fruit size and Miller and Hoffman (1988) advised an N/K ratio in the leaves of between 1.6 and 2.2 to achieve desired fruit size, with the K content higher than 0.9% and the N content higher than 1.8%. Both these elements have a rapid absorption rate and a high mobility in the vasculure system. Therefore foliar as well as soil applications of this element are successful in inducing a positive effect on fruit size (Lovatt, 2002).

The foliar application of a treatment consisting of these elements (5% KNO_3) and the synthetic auxin, 2,4-D, have a 25% increase effect on fruit size, compared to untreated trees (Erner et al., 1993). Corresponding results were obtained by Greenberg et al. (2006) with the combination of 2,4-D and the fertilizer 'Bonus-NPK' at concentration of 5% on 'Nova' mandarin in Israel.

2.2.4. Soil type

The effect of soil type on fruit size is a secondary effect, as the characteristics of the specific soil type determine the efficiency of cultural practices such as fertilization and irrigation, both factors affecting fruit size. Page et al. (1963) reported that the soil application of K in soils with a high cation exchange capacity are of little value, as these soils fix a large amount of

applied K. Lighter, sandy soils are thus better than heavy and saline soils i.e. inducing desired increase in fruit size (Gilfillan, 1987).

2.2.5. Water supply

Water management have a significant effect on rate of fruit growth and final fruit size (Gilfillan, 1987). The effect of water stress on fruit growth and fruit size are dependent on the time and duration of stress, with regard to the development stage of the fruit. Water stress during stage I of fruit development, reduce fruit set with insignificant reduction of fruit size (Hilgeman, 1977). Most of fruit size increase occur during stage II of fruit development (Bain, 1958) and water stress during this period, depending on the duration thereof, may lead to a decrease in growth rate and final fruit size (Hilgeman, 1977). Short periods of water stress during late spring and early summer reduce growth rate of fruit. However, increased growth rate of fruit, following irrigation after these short intervals lead to no significant effect on final fruit size (Cooper, 1963; Hilgeman, 1977). Prolonged periods of water stress during stage II of fruit development will lead to a reduction in final fruit size (Hilgeman, 1977).

2.2.6. Flower number and fruit load

A reduction in the number of flowers will reduce the eventual percentage fruit set, reducing the inter-fruit competition (Rabe and Van Rensburg, 1996) and increasing fruit size (Guardiola and García-Luis, 2000). Fruit from trees bearing small yields experience increased growth rate (Cooper et al., 1963). At high fruit loads increased inter-fruit competition for nutrients and water, especially during the early stages of fruit development, lead to a decrease in potential fruit size (Barry and Bower, 1997, Lenz and Cary, 1969). Measures aimed at

increasing the percentage fruit set, such as girdling or giberrellic acid sprays during flowering have been proven to lead to reduction in eventual fruit size, due to the increased inter-fruit competition for nutrients (Barry and Bower, 1997; Rabe et al., 1995).

2.3. USE OF SYNTHETIC AUXINS TO INCREASE FRUIT SIZE

Synthetic auxins are generally applied as fruit size enhancers in production areas or cultivars where fruit size is expected to be of unsatisfactory standards. Ensuring positive results by the application thereof are difficult, due to the interaction of concentration and timing of application with fruit load, and auxin type (Guardiola and García-Luis, 2000). Synthetic auxins follow different methods of action, determined mainly by the time of application (Guardiola, 1997).

2.3.1. Method of action of PGRs

Application during blossom

When applied during the flowering period, synthetic auxins increase the sink strength of selective young ovaries and cause an accelerated growth rate and eventual increase in fruit size (Guardiola and García-Luis, 1997; Guardiola and Lazaro, 1987; Ortola et al., 1997; Stewart et al., 1951). The increased growth rate obtained by treatments during flowering may lead to an eventual thinning of smaller fruitlets due to the increased growth rate and inter-fruit competition for assimilates (Guardiola and García-Luis, 1997).

Application prior end of physiological fruit drop

When synthetic auxins are applied during the period directly after bloom, increased fruit size is obtained due to the thinning of smaller fruit and resultant reduction in inter-fruit competition for carbohydrates and water (Guardiola, 1997; Stewart et al., 1951). During this stage, fruitlets are very sensitive to auxins and abscission of fruit is brought about by induced ethylene synthesis resulting from auxin applications (Guardiola, 1997).

Application after or at the end of physiological fruit drop

Application of synthetic auxins at later stage, at the end of physiological fruit drop and at the onset of cell enlargement stage, lead to the increased sink strength of remaining fruit and an increase in fruit size, without a significant thinning effect (Borroto et al., 1981; El-Otmani et al., 1993, El-Otmani et al., 1996; Guardiola and García-Luis, 2000; Guardiola, 1997, Rabe et al., 1995). For these treatments to be successful, the correct timing is critical, as fruitlets become insensitive to synthetic auxins as development progress and a too early application may lead to a significant reduction in the number of fruit (Duarte et al., 2006; Guardiola and García-Luis, 1997).

2.3.2. Factors determining success of the foliar application of PGR's

Concentration

Stewart et al. (1951) reported an increase in fruit size as concentration of auxin applications at full bloom increased, indicating a linear relationship between fruit size and auxin concentration. The phenological stage of fruit development, however, predominantly determine the concentration required to achieve desired fruit size increase, as fruit become less sensitive to applied synthetic auxins (Coggins and Hield, 1968; Koch, 1995; Rabe et al.,

1995), more particularly so, after physiological fruit drop (Guardiola, 1997; Koch, 1995; Ortola et al., 1997). Application of synthetic auxins at low concentrations during this phenological stage has limited effect on fruit size.

There is, however, an appropriate optimum concentration and a spray concentration beyond this level may negatively influence fruit qualities and vegetative growth (Stewart et al., 1951), with reports of enlarged oil glands in fruit rinds (Stewart and Hield, 1950), increased rind coarseness (Mupambi, 2010) (Fig. 2.5 A), increased rind thickness (Coggins and Hield, 1968, Saleem et al., 2007) and curling and wilting of young, vegetative growth (epinasty) (Stewart et al., 1951) (Fig. 2.5 B). Depending on the auxin type, with an increase in concentration thinning of fruit is expected (Guardiola and Lazaro, 1987; Greenberg et al., 2006).

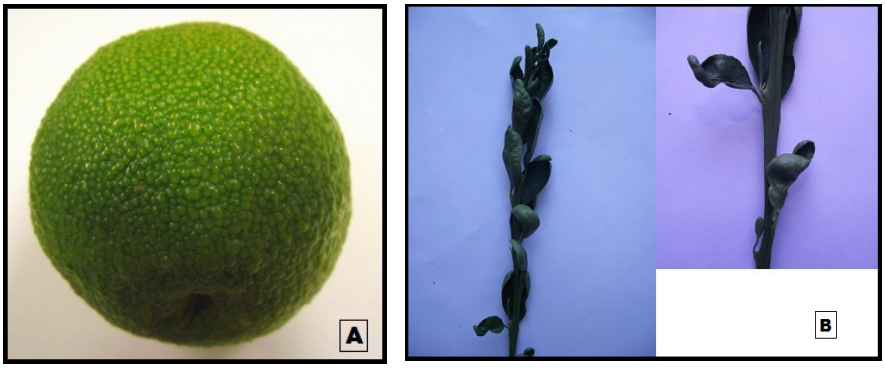

Fig. 2.5 A. Increased rind coarseness due to incorrect (too high) 2,4-D concentration on ‘Marisol’ Clementine. B. Curling of young vegetative growth due to 2,4-D application.

Fruit load

In years of excessive fruit load, fruit size is limited by the supply of water and assimilates due to high inter-fruit competition. A reduction in fruit sensitivity to applied auxins occurs. The

direct effect of auxins on sink strength of developing fruit, especially when applied at the end of physiological fruit drop, are enhanced in years of low fruit load when growth is generally not limited by assimilate supply (Guardiola and García-Luis, 1997).

Auxin type

Different auxin types have different methods of action and are thus applied at different timings and concentration, according to the desired effect (thinning or direct enhancing of fruit growth). As described, application of certain synthetic auxins prior to the end of physiological fruit drop induce the synthesis of ethylene by the fruit and lead to the abscission of smaller fruit (thinning). Phenoxy auxins, although also causing thinning of fruitlets, hardly induce ethylene synthesis, but rather delay abscission of fruitlets when applied at correct development stage and concentration (Borroto et al., 1981; Ortola et al., 1997; Shawki et al., 1978).

Different formulations of the same auxin may also differ in effectiveness, as in the case with the isopropyl ester formulation of 2,4-D, being more volatile than the dimethylamine salt and thus not recommended to be applied in close proximity to sensitive crops such as grapes (Anthony and Coggins, 1999).

2.4. FRUIT SIZE ENHANCING AUXINS COMMERCIALLY AVAILABLE

2.4.1. Corasil P® (2,4-dichlorophenoxy propionic acid) (Villa Crop protection)

The application of 2,4-DP prior to or during the period of physiological fruit drop achieves a thinning of smaller fruit and increase metabolite supply to remaining fruit (Duarte et al.,

2006; Rabe et al., 1995; Koch, 1995; Tuwana, 1999). However, it also has a direct stimulating effect on fruit growth if applied close to the end of physiological fruit drop period, without affecting fruit load (El-Otmani et al., 1993; El-Otmani et al., 1996; Koch, 1995; Rabe et al., 1995). This timing coincides with the period of cell enlargement, as described by Bain (1958) and application of 2,4-DP during this period (after physiological fruit drop) lead to increased fruit size due to stimulated cell enlargement, specifically of the pulp of the fruit (El-Otmani et al., 1993). It's effect on citrus cultivars are well-known to South African producers due to work by Koch (1995), Rabe et al. (1995) and Tuwana (1999), and a product, Corasil P® (Villa Crop protection) is commercially available to apply as fruit size enhancer.

2.4.2. Maxim® (3,5,6-trichloro-2-piridil oxyacetic acid) (Arysta LifeScience)

The application of 3,5,6-TPA have a significant increasing effect on the size of citrus fruit (El-Otmani et al., 1996; Guardiola and García-Luis, 2000; Greenberg et al., 2006). This formulation has a powerful thinning effect on young fruitlets due to induced ethylene synthesis and lead to unwanted fruit loss (Greenberg et al., 2006). Applications are therefore generally timed at the end of the physiological fruit drop period, to increase fruit size without unwanted fruit loss. The commercial product, Maxim® (Arysta LifeScience) is available and registered as fruit size enhancer in South Africa.

2.5. POTENTIAL OF 2,4-D (2,4-dichlorophenoxy acetic acid)

Initial research on 2,4-D reported fruit size increase with applications at blossom period (Stewart et al., 1951). Increase in fruit size obtained by 2,4-D at these timings, were mainly

due to delay in the maturation of developing fruit (Shawki et al., 1978; Stewart et al., 1951) and a direct growth effect by increasing the sink strength of fruit from treated trees (Duarte et al., 2006; Guardiola and Lazaro, 1987; Ortola et al., 1997). Stewart et al. (1951) observed an increase in fruit size of treated trees and reported an accompanied increase in fruit stem diameter and accelerated growth rate of tissues such as the rind, from trees treated at full bloom period.

The application of this phenoxy auxin at a later timing, when cell enlargement of the fruit predominates, leads to a direct stimulating effect on fruit growth and eventual size due to its capacity to enhance cell expansion and increase retention force of fruit (Agusti et al., 2002; Borroto et al., 1981). This was confirmed by Borroto et al. (1981) who reported a significant increase in fruit size of 'Valencia' orange with the application of 2,4-D at 10 and 20 $mg \cdot L^{-1}$ in mid-June (at end of physiological fruit drop) under tropical conditions, without reducing the number of fruit per tree. Erner et al. (1993) reported an increase in fruit size of 'Shamouti' and 'Valencia' oranges with the application of 20 $mg \cdot L^{-1}$ 2,4-D and 5% Bonus-NPK 6 to 8 weeks after full bloom. In recent studies, Greenberg et al. (2006) reported a significant increase in fruit size of 'Nova mandarin' hybrid with the application of 40 $mg.L^{-1}$ 2,4-D and 5% Bonus-NPK at fruit diameter of 13 mm. (end of physiological fruit drop). In addition, 2,4-D at 20 ppm combined with calcium chloride (0.5%) applied at fruit diameter of 15 mm. resulted in a significant increase in fruit size (Kassem et al., 2011).

2.5.1. Commercial products

There is currently no commercial product with 2,4-D as the active ingredient for fruit size enhancement in South Africa and the effect of various timings and concentrations of 2,4-D

treatments on fruit size of cultivars under South African conditions are not well documented. The isopropylester formulation of 2,4-D is registered as Alco® Citrus Fix™ in California and is commercially available as fruit size enhancer in 'Navel' and 'Valencia' orange as well as mandarin cultivars. Recommended timing of application for mandarins is 21 to 35 days after 75% petal drop and for 'Navel' and 'Valencia' orange between 6 and 19 mm. diameter, with increased concentrations at later timings.

2.5.2. Current possibility 2,4-D use in the South African citrus industry

Bloom application of 2,4-D successfully reduce the size of the Navel-end opening in oranges (Mupambi, 2010). This effect has various benefits, *viz.* a decrease in susceptibility to insect pests such as False Codling moth *Thaumatotibia (Cryptophlebia)leucotetra*. In addition, Mupambi (2010) reported a decrease in fruit size of 'Marisol' Clementine after application of 15 $mg \cdot L^{-1}$ and 25 $mg \cdot L^{-1}$ at full bloom and 100% petal drop. However, no significant effect was reported on navel oranges under South African conditions. A similar preliminary experiment to this study on the same cultivar and at similar timings, but at a lower concentration (10 $mg \cdot L^{-1}$), although not significantly, increased fruit size of treated fruit. Effectiveness of 2,4-D application at later timings are, however, are unknown.

2.6. CONCLUSSION

A variety of factors both controllable and uncontrollable, influence and may lead to unsatisfactory fruit size. Many cultivars, especially those of mandarin, genetically produce smaller fruit. Most of these cultivars are weakly parthenocarpic and fruit-setting measures

during flowering, such as GA_3 applications and girdling lead to a higher fruit set percentage and increased fruit load with small fruit as a result.

Synthetic auxins, such as Maxim® and Corasil P® are commercially available and generally applied during or after the physiological fruit drop period to increase fruit size. In many citrus producing countries, the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) is registered as fruit size enhancing product and achieve successful fruit size increase on a wide variety of cultivars. Recent work by Greenberg et al. (2006) and Kassem et al. (2011) reported significant fruit size increase with a treatment consisting of 2,4-D and K or Ca at a later timing, after blossom and petal drop.

The effect of this auxin on fruit size under South African conditions are not well studied and no commercial product of 2,4-D is registered specifically for fruit size enhancement. In recent studies, Mupambi (2010) reported a reduction in fruit size of 'Marisol clementine' mandarin with the application of 15 $mg.L^{-1}$ and 25 $mg.L^{-1}$ at full bloom and 100% petal drop and no significant effect of similar treatments on 'Navel' orange. These results serve as the starting point for this study.

In our study the effect of different late timings (after physiological fruit drop) of 10 $mg{\cdot}L^{-1}$ 2,4-D on fruit size, either alone or in combination with K and Ca will be evaluated. Two different formulations of 2,4-D, isopropyl ester and the less volatile dimethylamine salt will be used at separate sites and the effect thereof evaluated. The treatments' effects on internal and external qualities of treated fruit will also be evaluated to acquire more insight into the auxin's effect, with the objective of providing producers with an alternative measure to increase fruit size in problematic production areas and cultivars.

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3. PAPER 1: FOLIAR 2,4-DICHLOROPHENOXY ACETIC ACID (2,4-D) APPLICATION AFTER PHYSIOLOGICAL FRUIT DROP REDUCES FRUIT SPLITTING AND INCREASES FRUIT SIZE IN MANDARIN

Abstract

Citrus fruit splitting entails the initiation of cracks at the stylar- or navel-end of the fruit, developing into splitting and eventual abscission of affected fruit. Although cultural and environmental factors contribute to the susceptibility of fruit to the disorder, split-prone cultivars develop a genetically weak and split-susceptible rind. Foliar applications of different combinations of 2,4-dichlorophenoxy acetic acid (2,4-D), gibberellic acid (GA_3) and mineral nutrients calcium (Ca) and potassium (K) to reduce the disorder in split-prone mandarin cultivars, were evaluated over two consecutive growing seasons, 2010/2011 and 2011/2012. Studies were carried out on three different mandarin cultivars, viz., Marisol Clementine, Mor and Orri mandarin, at three different localities, viz., Citrusdal, De Doorns and Paarl, South Africa. Treatments were applied directly after physiological fruit drop (November/December) and at two later dates in summer (January and February). The application of 10 mg·L^{-1} 2,4-D directly after physiological fruit drop, either single or in combination with K increased rind thickness and reduced splitting of 'Marisol Clementine' mandarin and 'Mor' mandarin by up to 50% in both seasons. Post physiological fruit drop application of 10 mg·L^{-1} 2,4-D significantly increased growth rate (mm/day) of all the cultivars, resulting in bigger fruit at time of harvest and had no effect on rind coarseness and no styles remained attach. Except for a slight reduction in juice content (%) and total acidity (TA), there were no effects on the °Brix, and °Brix:TA at commercial harvest. A medium cover foliar spray of 10 mg·L^{-1} 2,4-D directly after physiological fruit drop is recommended for 'Marisol Clementine' mandarin and 'Mor' mandarin orchards with a history of severe fruit splitting and after excessive fruit set.

Additional keywords: *Citrus, physiological disorder, physiological fruit drop, rind coarseness, synthetic auxin, calcium, potassium*

3.1. Introduction

Fruit splitting or –cracking has been reported for almost every horticultural crop of economic importance, most notably apple [*Malus domestica* (Borkh.)] (Visai et al., 1989), apricot (Benson, 1994), cherry [*Prunus avium* (L.)] (Belmans and Keulemans, 1996), grape [*Vitis vinifera* (L.)] (Considine and Kriedmann, 1972), nectarine [*Prunus perisica* (L.)] (Gibert et al., 2007), prune [*Prunus domestica* (L.)] (Milad and Shackel, 1992) and tomato [*Lycopersicon esculentum* (Mill.)] (Peet, 1992). Although similarities in certain causal factors as well as certain aspects of the physiological development of the phenomena exists between these crops and citrus fruit, unique anatomical features of citrus fruit as well as difference in physiology of fruit development separates the phenomena in citrus from other crops.

Citrus fruit splitting is a physiological disorder caused by pressure resulting from the expanding pulp of an individual fruit on the rind (Almela et al., 1994; Barry and Bower 1997; Bower et al., 1992), eventually leading to a fissure in the rind at the stylar- or navel-end, and the splitting and premature drop of the fruit. Most important causal factors include insufficient nutrition, specifically of calcium (Ca) and potassium (K) (Bar-Akiva, 1975; De Cicco et al., 1988; Erickson, 1957), warm and humid climatic conditions (Almela et al., 1994; Barry and Bower, 1997; Rabe et al., 1990), irregular water supply (De Cicco et al., 1988) and heavy crop load (Barry and Bower 1997; Rabe et al., 1990).

Not only does splitting lead to an unwanted reduction in crop load, but also requires additional labour to sanitise orchards as split fruit provide perfect conditions for insect pest and decay development. A commercial solution is therefore required to control fruit splitting in South Africa. Foliar application of plant growth regulators (PGR's), the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) (Greenberg et al., 2006; Mupambi, 2010) and gibberellic acid (GA_3) (Almela et al., 1994; García-Luis et al., 1994; Rabe et al., 1990), either alone or in combination with mineral nutrients, such as Ca (Almela et al., 1994; Barry and Bower, 1997; Sdoodee and Chiarawipa, 2005) and K (Borroto et al., 1981; Greenberg et al., 2006), reduce the disorder.

In a recent study on 'Marisol Clementine' mandarin, 2,4-D- foliar applications of 15 $mg{\cdot}L^{-1}$ and 25 $mg{\cdot}L^{-1}$ at full bloom and petal drop, significantly reduced the disorder. However, the positive effect on reducing fruit splitting was accompanied by a negative effect on rind coarseness and also resulted in fruit with styles remaining attached until harvest (Mupambi, 2010), probably indicative of ineffective timing of treatment and/or wrong 2,4-D concentration.

The aim of this study is to evaluate the effect of different timings and a lower concentration of 2,4-D foliar applications, to significantly reduce the disorder on mandarin cultivars prone to fruit splitting, while maintaining acceptable fruit quality. The effect of 2,4-D foliar applications applied alone after physiological fruit drop, on fruit size, is also evaluated. It is hypothesised that foliar application of 10 $mg{\cdot}L^{-1}$ 2,4-D after physiological fruit drop, either alone or in combination with GA_3, Ca or K, may reduce the severity of the disorder of citrus fruit splitting, without compromising fruit quality and tree health.

3.2. Materials and Methods

3.2.1. Plant material and treatments

'Marisol Clementine' mandarin, Paarl

2010/11. The experiment was conducted in Paarl (33°69'S 18°95'E), South Africa on 'Marisol Clementine' mandarin trees, grafted on 'Carrizo citrange' (*C.sinensis* [L.] Osbeck × *P. trifoliata* [L.] Raf.) rootstock. The orchard was planted in 1994, at a spacing of 4.8 m between rows and 2 m between trees in a North-South row direction. Treatments were applied as foliar applications directly after physiological fruit drop (23 November 2010) when average fruitlet diameter was 14 mm, as well as at two later dates, 17 January 2011 (±35 mm) and 16 February 2011 (±43 mm) as summarised in Table 3.1.

2011/12. The same orchard was used for the experiment as in 2010/11. Treatments as indicated in Table 3.1 were applied only directly after physiological fruit drop (25 November 2011) at an average fruitlet diameter of 13 mm.

'Marisol Clementine' mandarin, Citrusdal

2010/11. The experiment was conducted in Citrusdal (32°51'S 19°51'E), South Africa, on 'Marisol Clementine' mandarin trees, grafted on 'Carrizo citrange' rootstock. The orchard was planted in 1993, at a spacing of 5 m between rows, 2.5 m between trees and a North-South row direction. Treatments were applied as foliar applications directly after physiological fruit drop (6 December 2010) with an average fruitlet diameter of 17 mm, as well as at two later dates, 13 January 2011 (±33 mm) and 14 February 2011 (±41 mm) as summarised in Table 3.1.

2011/12. The same orchard was used for the experiment as in 2010/11. Treatments as indicated in Table 3.1 were applied directly after physiological fruit drop (1 December 2011) at an average fruitlet diameter of 16 mm.

'Mor' mandarin* (*Citrus reticulata* Blanco), *Paarl

2010/11. The experiment was conducted in Paarl (33°83'S 18°98'E), South Africa on 'Mor' mandarin trees, grafted on 'Carrizo citrange' rootstock. The orchard was planted in 2000, at a spacing of 5 m between rows, 2 m between trees and in an East-West row direction. Treatments were applied as foliar applications directly after physiological fruit drop (17 December 2010) when the average fruitlet diameter was 15 mm, as well as at two later dates, 17 January 2011 (±25 mm) and 16 February 2011 (±35 mm) as summarised in Table 3.1.

2011/12. The same orchard and trees were used for the experiment as in 2010/11 and the same treatments as in the previous season were applied after physiological fruit drop (25 November 2011) at an average fruitlet diameter of 11 mm and on 15 January 2012 (±22 mm) and 16 February 2012 (±31 mm).

'Orri' mandarin, De Doorns

2010/11. The experiment was conducted in De Doorns (33°51'S 19°51'E), South Africa on 'Orri' mandarin trees, grafted on 'Carrizo citrange' rootstock. The orchard was planted in 2001, at a spacing of 5 m between rows, 2 m between trees and in a North-South row direction. Treatments were applied as foliar applications directly after physiological fruit drop (22 December 2010) when the average fruitlet diameter was 13 mm, as well as at two later

dates, 19 January 2011 (±22 mm) and 16 February 2011 (±30 mm) as summarised in Table 3.1.

3.2.2. Spray material and application method

2010/11: Two different formulations of 2,4-D (Dow AgroSciences (Pty.) Ltd.) were used. The less volatile dimethyl amine salt (10 mg·L^{-1}) was used on 'Marisol Clementine' mandarin in Paarl, as well as on 'Mor' and 'Orri' mandarin to avoid any harmful drift of applications onto other sensitive crops such as grapes. The more volatile iso-octyl ester was used on 'Marisol Clementine' mandarin in Citrusdal. Bonus-NPK (Haifa Chemicals Ltd) at 5% was applied both on its own and in a tank-mix with 2,4-D in all the experiments. $Ca(NO_3)_2$ (Omnia Nutriology) was applied on its own and in a tank-mix with 2,4-D on 'Marisol Clementine' mandarin in Paarl, as well as 'Mor' and 'Orri' mandarin. Gibberellic acid (GA_3) [ProGibb®, Philagro SA (Pty) Ltd], on its own and in a tank-mix with 2,4-D, was used on 'Marisol Clementine' mandarin in Citrusdal. A non-ionic wetting agent (Break-Thru®, Villa Crop Protection) with the active ingredient polyether-polymethylsiloxanecopolymer (1000 g·L^{-1}) was added to the spray solution at a rate of 5 ml per 100 L of spray solution in all the treatments that included 2,4-D and GA_3. Applications were made using a hand gun sprayer until runoff, at approximately 10 L per tree. For all the control treatments, trees were sprayed directly after physiological fruit drop with water and the non-ionic wetting agent.

2011/12: On 'Marisol Clementine' mandarin in Paarl, Bonus-NPK was applied at two concentrations (2% and 5%) both on its own and in combination with 2,4-D dimethyl amine salt (10 mg·L^{-1}). A single application of 2,4-D at 10 mg.L^{-1} was also included as treatment. On 'Marisol Clementine' mandarin in Citrusdal, 2,4-D iso-octyl ester was applied on its own,

as well as in combination with different commercial K products, *viz.,* 2% K-MAX [Nulandis (Pty) Ltd], 0.15% Carbology K (28.4% active K) [Terason (Pty) Ltd], 0.20% Quatro optifruit (28.4% active K) [Terason (Pty) Ltd] as well as 5% Bonus-NPK (38.5% active K) (Haifa Chemicals Ltd.). For 'Mor' mandarin, the same chemicals and method of application were used as in 2010/11. For all the control treatments, trees were sprayed directly after physiological fruit drop with water and the non-ionic wetting agent.

3.2.3. Experimental Design

Each experiment was carried out in a commercial orchard where standard production practices were utilised. In all the experiments, each treatment was repeated as ten single tree replicates in a randomized complete block design with buffer trees between treated trees as well as buffer rows. Only healthy uniform trees were used. Experiments were conducted in the growing seasons of 2010/11 and 2011/12 for 'Marisol Clementine' and 'Mor' mandarin. For 'Orri' mandarin only one experiment was conducted in 2010/11, as fruit set was too low in 2011/12 in this alternate bearing prone cultuvar.

3.2.4. Data collection and evaluations

Orchards were monitored for initiation of fruit splitting and split fruit were removed and counted at two- to three-weekly intervals from initiation of split (start of February) until harvest. Splitting is expressed as the total number split fruit removed and counted per tree and not as the percentage of the total fruit, as the total yield per tree was not determined in the experiment. In all the experiments, 10 fruit were randomly selected and tagged on 2,4-D replicates after chemical treatments was applied. The diameters of the tagged fruit were measured at monthly intervals until harvest. At harvest the growth rate (mm/day) of each fruit

were calculated by subtracting the initial diameter from the final diameter and dividing it by the number of days from initial measurement until final measurement. At commercial harvest, 12 fruit were sampled per tree, 6 from each row-side and from all sectors of the tree, to evaluate the treatment effect on important parameters of internal and external fruit quality as described below.

For external qualities, fruit rind colour was determined using the CRI colour chart for soft citrus no. 36 [Citrus Research International (CRI), 2004; Appendix 1a] with 8 being dark green and 1 a 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). An electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) was used to measure fruit diameter, fruit length and pedicel diameter.

Fruit were cut into half along the longitudinal plane for internal quality determinations. An electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) was used to measure rind thickness for each fruit at the equatorial region and stylar-end. The fruit were then juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth and the juice content (%) was determined by dividing the weight of the juice by the total fruit weight. °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 °Brix:TA ratio was calculated by dividing the °Brix values by the TA values.

3.2.5. Statistical analysis

Statistical analyses of variance (ANOVA) were performed using PROC GLM of the SAS Programme (version 9.1, SAS Institute Inc., Cary, NC, USA). Mean separation was done by the least significant difference (LSD) test where applicable ($P = 0.05$).

3.3. Results

3.3.1. 2010/11

'Marisol Clementine' mandarin, Paarl

Fruit Splitting: The total number of split fruit was significantly reduced in comparison to the control by the tank-mix application of 2,4-D and 5% Bonus-NPK just after physiological fruit drop (Table 3.2). Although all the other treatments failed to reduce the total number of split fruit per tree significantly, splitting was reduced by 2,4-D applied on its own, directly after physiological fruit drop, while the single application of $Ca(NO_3)_2$, as well as the January application of 2,4-D resulted in a tendency to increase fruit splitting. This trend was generally visible throughout the season as splitting was monitored (Table 3.2).

Fruit size: The application of 2,4-D after physiological fruit drop, increased fruit growth rate (mm/day) and fruit diameter significantly, in comparison to control. The later applications of 2,4-D had no significant effect, in comparison to the control (Table 3.3).

Fruit quality: Application of 2,4D after physiological fruit drop increased fruit diameter, but had no effect on fruit length, rind colour, and rind coarseness (Table 3.4). Rind thickness at

the stylar-end was not affected by any of the treatments, while rind thickness at the equatorial region was decreased by $Ca(NO_3)_2$. Pedicel diameter was reduced by 2,4-D application in January, as well as the tank-mix application of 2,4-D and $Ca(NO_3)_2$ (Table 3.4). There were no significant differences in the °Brix, TA, °Brix:TA ratio and the juice content (%) between treated or the control fruit (Table 3.5).

'Marisol Clementine' mandarin, Citrusdal

Fruit splitting: In 2011 there was a very low occurrence of splitting in this orchard, although application of 2,4-D directly after physiological fruit reduced splitting, it was not significant (Table 3.2).

Fruit size: None of the 2,4-D treatments had a significant effect on fruit growth rate ($mm.day^{-1}$) (Table 3.3).

Fruit quality: The treatments containing K, both alone and in combination with 2,4-D increased fruit diameter and Bonus-NPK on its own also fruit length (Table 3.6). There was no effect of treatments on rind colour and -coarseness, and rind thickness at the stylar-end or equatorial region. Pedicel diameter was increased by all treatments ecept the late 2,4-D and 2,4-D and $Ca(NO_3)_2$ application. There was no effect of treatments on internal fruit quality (Table 3.7).

'Mor' mandarin, Paarl

Fruit splitting: The 'Mor' orchard experienced a severe 'off'-year and both fruit load (data not shown) and splitting were very low in 2011 (Table 3.2). Although 2,4-D on its own and tank-mix with Bonus-NPK reduced splitting, none of the treatments resulted in a significant reduction compared to the control (Table 3.2).

Fruit size: Treatment with 2,4-D directly after physiological fruit drop significantly increased fruit growth rate ($mm.day^{-1}$), in comparison to the control (Table 3.3).

Fruit quality: Both 2,4-D on its own, as well as tank-mix with Bonus-NPK had a pronounced effect on fruit size, with both fruit diameter and length significantly higher than control (Table 3.8). In addition, treatments increased pedicel diameter, as well as rind thickness at the stylar-end of the fruit. The °Brix was not affected by the treatments, but the TA of fruit treated with a tank-mix of 2,4-D and Bonus-NPK was significantly lower and as a result, an increase in °Brix:TA ratio occurred (Table 3.9). TA of trees treated with 2,4-D alone was also reduced, but to a lesser extent and therefore did not affect the °Brix:TA ratio. Both applications reduced juice content (%) of the fruit, but 2,4-D after physiological fruit drop more so than the tank-mix (Table 3.9).

'Orri' mandarin, De Doorns

Fruit splitting: None of the treatments had a significant effect on splitting (Table 3.2).

Fruit size: Treatment with 2,4-D directly after physiological fruit drop significantly increased fruit growth rate (mm/day), in comparison to the control (Table 3.3).

Fruit quality: Treatments did not influence fruit dimensions (diameter and length), rind colour and -coarseness (Table 3.10). All the treatments except $Ca(NO_3)_2$ increased rind thickness relative to the control at the stylar-end of the fruit, while equatorial region rind thickness increased following a tank-mix of 2,4-D and 5% Bonus-NPK. The pedicel diameter of fruit from 2,4-D treated trees in February, were significantly the lowest while no other treatment differed from the control (Table 3.10). The °Brix and °Brix:TA ratio of fruit treated with tank-mix of 2,4-D + Bonus-NPK were significantly higher than control fruit (Table 3.11). There were no significant difference in the juice content (%) between treated and control fruit (Table 3.11).

3.3.1. 2011/12

'Marisol Clementine' mandarin, Paarl

Fruit splitting: All the treatments significantly reduced the total number of split fruit in comparison to the control, with tank-mix application of 2,4-D and 5% Bonus-NPK the most successful although not significantly more so than the other treatments (Table 3.12). This trend could be seen throughout the season from early February.

Fruit size: Application of 2,4-D increased fruit growth rate ($mm.day^{-1}$) (Table 3.13), as well as fruit diameter and fruit length (Table 3.14).

Fruit quality: Application of 2,4-D alone as well as in tank-mix with 5% Bonus-NPK increased fruit diameter, while all the treatments, except the application of 2.5% Bonus-NPK on its own, increased fruit length (Table 3.14). All the treatments resulted in significantly greener fruit and none of the treatments had a significant effect on rind coarseness (Table

3.14). Application of 5% Bonus-NPK increased the pedicel diameter, while all the treatments except 2.5% Bonus-NPK resulted in a reduction in the pedicel diameter compared to the control (Table 3.14). All the treatments increased fruit rind thickness at the stylar-end except 5% Bonus-NPK, also at the equatorial region of the fruit except the tank-mix of 2,4-D and 2.5% Bonus-NPK (Table 3.14). There were no differences in the °Brix, TA and °Brix:TA ratio, while the juice content (%) of fruit from trees treated with a tank-mix application of 2,4-D and 2.5% Bonus-NPK was significantly lower than fruit from control trees (Table 3.15). Fruit from 2,4-D treated trees had a lower juice content (%), than fruit from trees treated with 2.5% or 5% Bonus-NPK.

'Marisol Clementine' mandarin, Citrusdal

Fruit splitting: All the treatments reduced total fruit splitting significantly compared to the control (Table 3.12). There were no significant differences between different K-treatments, and the combination with 2,4-D had similar response as 2,4-D applied on its own.

Fruit size: Treatment with 2,4-D directly after physiological fruit drop significantly increased fruit growth rate ($mm.day^{-1}$), in comparison to the control (Table 3.13).

Fruit quality: There was no significant effect on fruit size, and rind coarseness and -colour of treated fruit (Table 3.16). Pedicel diameter was reduced except by 2,4-D + KMAX. Rind thickness was increased at the stylar-end by 2,4-D + Quatro optifruit while no significant differences were found in the equatorial region. TA did not differ significantly between treatments and the control fruit, but some slight differences were found between treatments (Table 3.17). The treatments had no effect on the other internal fruit quality parameters (Table 3.17).

'Mor' mandarin, Paarl

Fruit splitting: Post physiological fruit drop application and January application of 2,4-D on its own, as well as tank-mix with 5% Bonus-NPK and 2% $Ca(NO_3)_2$ significantly reduced splitting compared to the control treatment (Table 3.12). The positive impact of 2,4-D was reduced if applied in January and not significant when delayed until February (Table 3.12).

Fruit size: Treatment with 2,4-D directly after physiological fruit drop significantly increased fruit growth rate ($mm.day^{-1}$), in comparison to the control (Table 3.13).

Fruit quality: All the 2,4-D treatments applied after physiological fruit drop, increased both fruit diameter and length (Table 3.18). There were no effects of treatments on rind colour, rind coarseness or pedicel diameter. All the treatments with Ca or K, as well as tank-mix with 2,4-D, significantly increased rind thickness at the stylar-end, while 2,4-D applied alone, directly after physiological fruit drop, increased rind thickness at the equatorial region of the fruit (Table 3.18). All the treatments, except for the application of 2,4-D after physiological fruit drop, reduced the juice content (%) of treated fruit, while there was no effect of any of the treatments on TA, °Brix and °Brix:TA ratio (Table 3.19).

3.4. Discussion

Foliar application of 10 $mg{\cdot}L^{-1}$ 2,4-D directly after physiological fruit drop reduced splitting of 'Marisol Clementine' as well as 'Mor' and 'Orri' mandarin. This is in agreement with Greenberg et al. (2006) who reported split reduction in 'Nova' mandarin albeit with foliar application of 40 $mg{\cdot}L^{-1}$ 2,4-D at an average fruitlet diameter of 13 mm (after physiological fruit drop), while Almela et al. (1994) reported successful reduction of splitting of 'Nova'

mandarin with two applications of a combination of 2,4-D and GA_3 (20 mg·L^{-1}), 30 and 60 days prior to split initiation.

However, treatment effect on fruit quality parameters such as rind coarseness was not evaluated in previous studies. The use of 2,4-D as a herbicide is common practice to control broadleaf weeds. It is rapidly absorbed by roots, stems and leaves and translocated in the phloem to young meristematic tissue (Ashton et al., 1991). It accumulates in sink organs such as young leaves, flowers or fruitlets where it stimulates cell expansion (Mitchell, 1961). Pre-harvest treatment with 2,4-D in citrus at very high concentrations (>20 mg·L^{-1}) lead to the development of excessively thick and coarse rinds of treated fruit due to enlarged oil glands in the flavedo (Stewart et al., 1951). Recently, Mupambi (2010) reported 2,4-D applications of 25 mg·L^{-1} at full bloom, as well as 15 mg·L^{-1} and 25 mg·L^{-1} at petal drop reduced splitting in 'Marisol Clementine' mandarin. However, these treatments also increased rind coarseness and the styles failed to abscise on treated fruit, remaining attached until harvest (Mupambi, 2010). Styles remaining attached until harvest could be explained by the effect of auxins blocking the capacity of ethylene to stimulate the natural abscission of plant material (Goren, 1993). Normally, cellulase breaks down the vascular bundles connecting the style to the fruit, leading to the formation of the abscission layer which is visible as the stylar-scar after style abscission. In addition to rind coarseness and attached styles, Mupambi (2010) reported malformation of young leaves on new growth flushes due to application of 2,4-D during bloom. However, with the concentration and time of 2,4-D application used in this study, no new growth flushes with young growing leaves were present and thus, concurring with Coggins and Hield (1968), treatments had no visible effect on vegetative growth and tree health.

After physiological fruit drop, stage II of fruit development begins during which cell enlargement takes place and in which cell growth of the pulp and albedo as well as a low level of cell division in the flavedo occurs (Bain, 1958). Application of 10 $mg \cdot L^{-1}$ 2,4-D at this stage of fruit growth seems to be optimal to reduce fruit splitting. Application of 2,4-D in this period not only encouraged cell growth of the rind, but also allowed for successful natural stylar abscission to occur, resulting in no styles remaining attached and no significant effect on rind coarseness. Treatments of 2,4-D later than December were unsuccessful in reducing fruit splitting in 'Marisol Clementine' mandarin as well as 'Mor' mandarin. January and February application of 10 $mg \cdot L^{-1}$ 2,4-D in some instances increased the number of split fruit, indicating a decrease in fruit sensitivity to synthetic auxins as fruit development progressed (Duarte et al., 2006).

Application of either 2.5% or 5% Bonus-NPK resulted in split reduction of 'Marisol Clementine' mandarin in the 2011/12 season. Potassium plays a key role in growth processes of plant cells. An optimum K-content is critical in maintaining cell osmotic potential and turgor pressure, which is essential for cell growth (Leigh and Wyn Jones, 1984). With a decrease in plant K-status or availability, K from the cytoplasm is utilized, resulting in a decline in cell growth (McAfee, 2001). In citriculture, supplementing trees with K, increases both leaf and rind K concentration, resulting in bigger fruit with thicker rinds, which have been shown to reduce the susceptibility of fruit to split (Bar-Akiva, 1975; De Cicco et al., 1988; Koo, 1961). Combining K with synthetic auxins, improves its effect, both as a fruit size enhancer (Erner et al., 1993) as well as to reduce fruit splitting (Greenberg et al., 2006). Treatment of 10 $mg \cdot L^{-1}$ 2,4-D in combination with potassium (K), increased rind thickness of all the mandarin cultivars in our study and led to a reduction of fruit splitting in both 'Marisol Clementine' mandarin and 'Mor' mandarin. This decrease in split with the addition of K

concurs with Greenberg et al. (2006) who reported a reduction in splitting of 'Nova' mandarin with the combined foliar application of 40 $mg \cdot L^{-1}$ 2,4-D and 5% Bonus-NPK after physiological fruit drop. Stimulation of cell expansion by 2,4-D treatments (Mitchell, 1961) on its own, as well as in some instances in combination with K, may have been responsible for the increase (not always statistically significant) in rind thickness at the stylar-end and equatorial region of treated fruit. On 'Marisol' in Paarl, addition of K improved the effect of 2,4-D, but in Citrusdal, addition of K had a similar response as 2,4-D on its own. This may be due to a lower K-status in trees in Paarl (data not shown).

Foliar applications of $Ca(NO_3)_2$ were ineffective in reducing splitting. This contradicts reports of split reduction with two applications of 2% $Ca(NO_3)_2$ 30 and 60 days before split initiation on 'Nova' mandarin (Almela et al., 1994). Sdoodee and Chiarawipa (2005) also reported successful reduction of splitting of 'Shogun' mandarin with consecutive, monthly foliar applications of 1% $CaCl_2$ starting four months after full bloom. Transport of Ca to developing fruit is a passive process, dependent on the flow of the transpiration stream through the plant. Very low to no translocation of Ca from old leaves or other plant organs to newly developing leaves, meristems and fruit takes place (Hanger, 1979). Therefore, young fruitlets will show very little response to only one foliar application of Ca, as reported in this study. The best strategy to improve fruit Ca is optimum root uptake and transport via the transpiration stream in the xylem (Hanger, 1979).

The high seasonal variability in severity between splitting in 2011 and 2012, especially in the mandarin cultivars, concur with Almela et al. (1994) and is evidence of the complexity of the disorder and the different causal factors. Mandarins are prone to alternate bearing and in years of heavy fruit load, a more severe incidence of % split fruit is likely to occur (Gilfillan

and Stevenson, 1984). With an increase in the number of fruit per tree, a linear increase in the inter-fruit competition for water and assimilates is expected. In 2011 the average number of split fruit on control trees of 'Mor' mandarin were 8 fruit per tree with the fruit load in the affected orchard at 18 ton/ha. In 2012 the average number split fruit per tree in the control increased to 31 fruit per tree with the fruit load in the affected orchard at 29 ton/ha. Lenz and Cary (1969) reported a decrease in fruit size and more importantly, a decrease in rind thickness of citrus fruit with an increased crop load per tree. As crop load increased, the K-concentration, a very important contributing agent to the development of a healthy and thick rind (Chapman, 1968), declined in both the leaves and shoots of citrus trees (Lenz, 2000). In all the cultivars, splitting was more severe in years with a reduced rind thickness (data not shown). Therefore, as seen in 'Marisol' in Paarl in 2012, supplementing the trees with K, may reduce the potential of an orchard to experience severe levels of fruit splitting, by increasing rind thickness.

Factors other than crop load were responsible for the high variability in splitting of 'Marisol' in Paarl, as the fruit load were similar in both years, but the average number split fruit in 2011 was 209 and in 2012, only 68. However, average rind thickness of fruit was lower in 2011 than in 2012, partly explaining the higher severity of the disorder in the particular year. This concurs with Almela et al. (1994), who reported an inverse relationship between rind thickness and fruit splitting, with an increase in number of split fruit as the average rind thickness decreased. Several environmental as well as cultural factors have an effect on rind thickness and a contributing, cumulative effect on the initiation and extent of severity of splitting. In years of very warm and humid conditions, splitting is more severe (Almela et al., 1994) due to the development of a thinner rind (Reuther et al., 1973). Mineral nutrient and

water stress are also contributing factors in splitting initiation and severity (De Cicco et al., 1988) due to their negative effect on rind thickness.

The foliar application of 10 mg·L^{-1} 2,4-D directly after physiological fruit drop in general, significantly increased the growth rate (mm.day^{-1}) and of all the mandarin cultivars evaluated. This resulted in bigger fruit at time of commercial harvest and concurs with previous reports in mandarins (El-Otmani et al., 1996; Greenberg et al., 2006). Treatment of 2,4-D after physiological fruit drop increased rind thickness of 'Mor' and 'Orri' mandarin in both seasons and in 'Marisol Clementine' mandarin cultivated in Paarl in 2011/12, but not in 2010/11 and not in Citrusdal. The rind contributes to the total weight of the fruit and as a result, increase in fruit size is expected with increase in rind thickness. However, January and February 2,4-D applications increased rind thickness of mandarin fruit accordingly, but had no significant effect on total fruit growth rate (mm.day^{-1}) as well as final fruit size. Application of 2,4-D after physiological fruit drop, in concurrence with El-Otmani et al. (1993), is thought to have also stimulated cell expansion in the juice vesicles in the pulp of treated fruit, thereby resulting in significantly larger fruit in comparison to treatments in January and February, as well as the control. Directly after physiological fruit drop, stage II of fruit development initiates and cell enlargement of the endocarp starts, while cell division of the rind continues until ripening period (Bain, 1958). In addition to increased sink strength of treated fruit, 2,4-D treatment can increase transport effectiveness of water, nutrients and assimilates by increasing the capacity of the vascular system that connects the source (leaves) to the sink (developing fruit) (Bustan et al., 1995). Mesejo et al. (2003) reported an increase in the pedicel diameter of mandarin resulting from an increase in the central xylem cylinder as well as the number and the average diameter of xylem vessels, after foliar application of 15 mg·L^{-1} 2,4-D after physiological fruit drop. The effect of foliar application of 2,4-D on

pedicel diameter was erratic. In 'Marisol Clementine' mandarin, pedicel diameter was generally reduced by 2,4-D treatments, while in 'Mor', 2,4-D treatments increased the pedicel diameter.

To conclude, foliar application of 10 mg·L^{-1} 2,4-D directly after physiological fruit drop reduced the number of split fruit in 'Marisol Clementine' by 42% in 2011 and 50% in 2012 with a slight increase in rind coarseness and no stylar attachment. In 'Mor' mandarin, the number of split fruit was reduced by 63% in 2011 and 50% in 2012, with no effect on rind coarseness and no styles remaining attached. Growth rate (mm/day) of 'Marisol Clementine' mandarin as well as 'Mor' and 'Orri' mandarin was increased by a foliar application of 10 mg·L^{-1} directly after physiological fruit drop. Successful increase in growth rate by 2,4-D application after physiological fruit drop is thought to be a result of a combination of (1) increase in rind thickness, as well as (2) increased expansion of juice vesicles of the pulp, due to an increase in the sink strength for water and assimilates of treated fruit. Except for reduced juice content (%) and TA in certain cultivars, there was generally no significant effect of the treatments on the °Brix, as well as °Brix:TA ratio. It is possible that the increase in rind thickness could negatively affect juice content (%) of the fruit, as rind weight is included in juice content (%) calculations. A single medium cover application of 10 mg·L^{-1} 2,4-D timed directly after physiological fruit drop on 'Marisol Clementine' and 'Mor' mandarin can be recommended in orchards with a history of severe fruit splitting, especially in years with heavy fruit set following physiological fruit drop.

3.5. References

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Table 3.1: Summary of foliar chemical applications indicating concentrations, timing, cultivars and experimental site.

Treatments	Average fruitlet diameter at time of foliar application (mm) n=100						
	'Marisol Clementine' mandarin Paarl		'Marisol Clementine' mandarin Citrusdal		'Mor' mandarin Paarl		'Orri' mandarin De Doorns
Year	**2010/11**	**2011/12**	**2010/11**	**2011/12**	**2010/11**	**2011/12**	**2010/11**
2.5% Bonus-NPK* [y]	-	13	-	-	-	-	-
5% Bonus-NPK*	14	13	17	16	15	11	13
2% $Ca(NO_3)_2$*	14	-	17	-	15	11	13
20 mg·L^{-1} GA_3 January	-	-	33	-	-	-	-
10 mg·L^{-1} 2,4-D[x]*	14	13	17	16	15	11	13
2,4-D January	35	-	33	-	25	22	22
2,4-D February	43	-	41	-	35	31	30
2,4-D and 2.5% Bonus-NPK*	-	13	-	-	-	-	-
2,4-D and 5% Bonus-NPK*	14	13	-	16	15	11	13
2,4-D + 2% $Ca(NO_3)_2$*	14	-	-	-	15	11	13
2,4-D + GA_3 January	-	-	33	-	-	-	-
2,4-D + 2% KMAX*	-	-	-	16	-	-	-
2,4-D + 0.15% Carbology K*	-	-	-	16	-	-	-
2,4-D + 0.20% Quatro optifruit*	-	-	-	16	-	-	-

[x] Two different 2,4-D formulations used: dimethyl amine salt (Paarl+De Doorns) and iso-octyl ester (Citrusdal)
[y] For detail on chemicals see section 3.2.2
*Applied directly after physiological fruit drop

Table 3.2: Effect of different combinations of chemical applications on total fruit splitting of various cultivars in the production season of 2010/11

Treatment	Ave. nr. split fruit per tree			
	'Marisol Clementine' mandarin Paarl	**'Marisol Clementine' mandarin Citrusdal**	**'Mor' mandarin Paarl**	**'Orri' mandarin De Doorns**
Control	209abc[z]	15[ns]	8ab[z]	22[ns]
2,4-D*	121 cd	12	3b	18
Bonus NPK*	154 bcd	13	5ab	20
$Ca(NO_3)_2$*	237 ab	-	9a	24
GA_3	.	15	-	-
2,4-D + Bonus NPK*	79 d	14	7ab	20
2,4-D + $Ca(NO_3)_2$*	151 bcd	14	5b	21
2,4-D January	273 a	16	8a	16
2,4-D February	184 bcd	21	7ab	19
P-value	*0.0038*	*0.1966*	*0.0140*	*0.4753*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

[ns] No significant differences

*after physiological fruit drop

Table 3.3: Effect of different application dates of 2,4-D on fruit growth rate of various mandarin cultivars in the production season of 2010/11

Treatment	Fruit growth rate (mm.day^{-1})			
	'Marisol Clementine' mandarin, Paarl	**'Marisol Clementine' mandarin, Citrusdal**	**'Mor' mandarin, Paarl**	**'Orri' mandarin, De Doorns**
Control	0.28b[z]	0.30[ns]	0.24b	0.30b
2,4-D*	0.30a	0.30	0.26a	0.32a
2,4-D January	0.27b	0.29	0.23c	0.31b
2,4-D February	0.27b	0.30	0.23c	0.30b
P-value	*0.0001*	*0.2101*	*0.0312*	*0.0441*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

[ns] No significant differences

*after physiological fruit drop

Table 3.4: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Marisol Clementine' mandarin, Paarl 2010/11.

Treatment	External fruit quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	--------(mm.)---------		(1 to 6)	--(1 to 4)--	----------(mm.)----------		
Control	53.10 b[z]	48.67 abc	5 [ns]	2[ns]	4.17 a	1.30[ns]	2.09ab
2,4-D*	55.30 a	49.84 a	5	2	4.05 abc	1.20	2.01abc
Bonus NPK*	53.63 b	48.96 abc	5	2	4.10 ab	2.02	2.15a
$Ca(NO_3)_2$*	53.65 b	49.29 abc	5	2	4.04 abc	1.20	1.84c
2,4-D + Bonus NPK*	56.16 a	49.68 a	5	2	4.15 a	1.16	2.01bc
2,4-D + $Ca(NO_3)_2$*	53.68 b	49.33 ab	5	2	3.98 bcd	1.25	2.00abc
2,4-D January	53.03 b	48.09 c	5	2	3.95 cd	1.11	1.93bc
2,4-D February	52.81 b	48.31 bc	4	2	4.08 ab	1.40	2.07ab
P-value	*0.0001*	*0.0492*	*0.1410*	*0.0941*	*0.0125*	*0.2895*	*0.0264*

[x] Equatorial region

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

[ns] No significant differences

*after physiological fruit drop

Table 3.5: Effect of different combinations of chemical applications on internal fruit quality of 'Marisol Clementine' mandarin fruit at commercial harvest, Paarl 2010/11.

Treatment	Internal quality			
	TA[x]	°Brix	°Brix:TA	Juice content (%)
Control	1.48[ns]	9.87[ns]	6.78[ns]	52.33[ns]
2,4-D*	1.34	10.11	7.65	49.29
Bonus NPK*	1.38	10.31	7.55	50.16
$Ca(NO_3)_2$*	1.34	9.91	7.49	52.07
2,4-D + Bonus NPK*	1.48	10.36	7.13	49.00
2,4-D + $Ca(NO_3)_2$*	1.38	10.26	7.53	52.03
2,4-D January	1.34	10.51	8.14	49.64
2,4-D February	1.34	10.48	7.91	50.48
P-value	*0.3678*	*0.4015*	*0.0557*	*0.6957*

[x] Titratable acidity
[ns] No significant differences
*after physiological fruit drop

Table 3.6: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Marisol Clementine' mandarin, Citrusdal 2010/11.

Treatment	External quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	--------(mm.)------		(1 to 6)	--(1 to 4)--	----------(mm.)----------		
Control	55.08c[z]	53.44b	6[ns]	3[ns]	3.99 c	1.44 [ns]	2.96 [ns]
2,4-D*	55.41c	53.20b	6	3	4.02 ab	1.47	2.36
Bonus NPK*	58.43a	56.98a	6	3	4.22 a	1.56	2.48
GA_3 January	55.62bc	53.62b	6	3	3.94 b	1.29	3.03
2,4-D + Bonus NPK*	57.56ab	54.84b	6	3	4.22 a	1.53	2.44
2,4-D + $Ca(NO_3)_2$*	55.96bc	53.97b	6	3	3.92 bc	1.55	2.37
2,4-D January	54.54c	53.01b	6	3	3.72 c	1.37	3.18
2,4-D February	54.33c	53.69b	6	3	3.81 bc	1.56	2.24
P-value	*0.0009*	*0.0095*	*0.0678*	*0.0879*	*0.0001*	*0.0800*	*0.3860*

[x] Equatorial region
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 3.7: Effect of different combinations of chemical applications on internal fruit quality of 'Marisol Clementine' mandarin fruit at commercial harvest, Citrusdal 2010/11.

Treatment	Internal quality			
	TA[x]	°Brix	°Brix:TA	Juice content (%)
Control	1.23 [ns]	11.04 [ns]	9.02 [ns]	44.47 [ns]
2,4-D*	1.23	11.15	9.14	44.76
Bonus NPK*	1.19	10.74	9.06	38.54
GA_3 January	1.27	11.16	8.77	43.90
2,4-D + Bonus NPK*	1.27	11.32	8.94	41.44
2,4-D + $Ca(NO_3)_2$*	1.31	11.34	8.81	43.85
2,4-D January	1.22	9.23	9.23	45.08
2,4-D February	1.23	10.97	9.01	42.93
P-value	*0.3290*	*0.2791*	*0.9114*	*0.1485*

[x] Titratable acidity
[ns] No significant differences
*after physiological fruit drop

Table 3.8: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Mor' mandarin, Paarl 2010/11.

Treatment	External quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	--------(mm.)-------		(1 to 6)	-(1 to 4)-	----------(mm.)----------		
Control	65.30 b[z]	50.75b	1[ns]	1[ns]	3.04 b	1.84 b	5.21 [ns]
2,4-D*	70.93 a	53.75a	2	1	3.87 a	2.18 a	5.21
2,4-D + Bonus NPK*	70.91 a	53.41a	2	1	3.64 a	2.16 a	5.51
P-value	*0.0001*	*0.0012*	*0.4571*	*0.2708*	*0.0001*	*0.0432*	*0.4325*

[x] Equatorial region
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 3.9: Effect of different combinations of chemical applications on internal fruit quality of 'Mor' mandarin fruit at commercial harvest, Paarl 2010/11.

Treatment	Internal quality			
	TA[x]	**°Brix**	**°Brix:TA**	**Juice content (%)**
Control	1.56 a[z]	13.39[ns]	8.62 b	52.52a
2,4-D*	1.43 b	13.11	9.18b	46.71c
2,4-D + Bonus NPK*	1.29 c	12.82	10.05a	50.82b
P-value	*0.0359*	*0.0912*	*0.1235*	*0.0145*

[x] Titratable acidity
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 3.10: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Orri' mandarin, De Doorns 2010/11.

Treatment	External quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	----------(mm.)----------		-(1 to 6)-	--(1 to 4)--	----------(mm.)----------		
Control	57.33 [ns]	47.58[ns]	2[ns]	1[ns]	4.68 ab[z]	2.18 c	6.22 bc
2,4-D*	59.65	49.13	2	1	4.63 ab	2.76 ab	7.46 abc
Bonus NPK*	58.37	48.56	1	1	4.61 b	2.59 b	7.21 bc
$Ca(NO_3)_2$*	58.86	48.17	2	1	4.60 b	2.20 c	4.98 c
2,4-D + Bonus NPK*	59.45	48.86	2	2	4.81 ab	2.60 b	12.52 a
2,4-D + $Ca(NO_3)_2$*	60.19	49.24	1	1	4.92 a	2.81ab	7.81 abc
2,4-D January	59.35	49.16	1	2	4.66 ab	2.90 a	10.41 ab
2,4-D February	57.95	48.16	1	1	4.29 c	2.80 ab	8.09 abc
P-value	*0.0771*	*0.6654*	*0.4420*	*0.8061*	*0.0122*	*0.0001*	*0.0124*

[x] Equatorial region
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 3.11: Effect of different combinations of chemical applications on internal fruit quality at harvest of 'Orri' mandarin, De Doorns 2010/11.

Treatment	Internal quality			
	TA[x]	°Brix	°Brix:TA	Juice Content (%)
Control	1.45 ab[z]	14.48 bc	10.11bc	47.71 [ns]
2,4-D*	1.34bc	14.60 b	10.93ab	47.06
Bonus NPK*	1.50a	14.59 b	9.83c	46.52
$Ca(NO_3)_2$*	1.34bc	14.59 b	10.91ab	46.93
2,4-D + Bonus NPK*	1.30bc	15.13 a	11.65a	47.14
2,4-D + $Ca(NO_3)_2$*	1.51a	14.68 ab	9.84c	45.71
2,4-D January	1.45ab	14.11 c	9.83c	43.02
2,4-D February	1.46ab	14.52 bc	10.07c	46.64
P-value	*0.0210*	*0.0151*	*0.0001*	*0.8640*

[x] Titratable aciditty
[z] Means with a different letter differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 3.12: Effect of different combinations of chemical applications on total fruit splitting of various cultivars in the production season of 2011/12.

Treatment	Ave. nr. split fruit per tree		
	'Marisol Clementine' mandarin Paarl	**'Marisol Clementine' mandarin Citrusdal**	**'Mor' mandarin Paarl**
Control	68 a[z]	54a	31a
2.5% Bonus-NPK*	42 b	-	-
5% Bonus NPK*	33 b	-	26a
2,4-D*	33 b	25cd	15c
2,4-D + 2.5% Bonus-NPK*	26 b	-	-
2,4-D + 5% Bonus- NPK*	18 b	28bcd	13c
2% $Ca(NO_3)_2$*	-	-	23ab
2,4-D + 2% $Ca(NO_3)_2$*	-	-	13c
2,4-D + GA_3 January	-	-	-
2,4-D + 2% KMAX*	-	33b	-
2,4-D + 0.15% Carbology K*	-	23d	-
2,4-D + 0.20% Quatro optifruit*	-	33b	-
2,4-D January	-	-	20b
2,4-D February	-	-	26a
P-value	*0.0021*	*0.0023*	*0.0001*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
*after physiological fruit drop

Table 3.13: Effect of different dates of 2,4-D applications on fruit growth rate of various mandarin cultivars in the production season of 2011/12.

Treatment	Fruit growth rate mm.day^{-1}		
	'Marisol Clementine' mandarin, Paarl	**'Marisol Clementine' mandarin, Citrusdal**	**'Mor' mandarin, Paarl**
Control	0.35b[z]	0.25b	0.20c
2,4-D*	0.37a	0.27a	0.22a
2,4-D January	-	-	0.21b
2,4-D February	-	-	0.21b
P-value	*0.0097*	*0.0401*	*0.0401*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
*after physiological fruit drop

Table 3.14: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Marisol Clementine' mandarin, Paarl 2011/12.

Treatment	External fruit quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	--------(mm.)---------		(1 to 6)	--(1 to 4)--	----------(mm.)----------		
Control	57.12dc[z]	51.76b	6a	3[ns]	3.27 b	1.50c	2.15d
2.5% Bonus-NPK*	56.50d	52.00b	7b	3	3.20 b	1.65b	2.32bc
5% Bonus NPK*	58.72bc	54.75a	7b	3	3.52 a	1.60bc	2.39ab
2,4-D*	61.79a	55.55a	7b	3	3.01 c	1.83a	2.47a
2,4-D + 2.5% Bonus-NPK*	58.49bc	54.12 a	7b	3	2.91 dc	1.74ab	2.23cd
2,4-D + 5% Bonus-NPK*	59.52b	54.5a	7b	3	2.78 d	1.70ab	2.34bc
P-value	*0.0001*	*0.0356*	*0.0068*	*0.1042*	*0.0109*	*0.0287*	*0.0103*

[x] Equatorial region

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

[ns] No significant differences

*after physiological fruit drop

Table 3.15: Effect of different combinations of chemical applications on internal fruit quality of 'Marisol Clementine' mandarin fruit at commercial harvest, Paarl 2011/12.

Treatment	Internal quality			
	TA[x]	°Brix	°Brix:TA	Juice content (%)
Control	1.46 [ns]	10.93 [ns]	7.68 [ns]	49.44 ab[z]
2.5% Bonus-NPK*	1.46	11.26	7.94	49.10 ab
5% Bonus NPK*	1.26	10.91	8.73	50.08 a
2,4-D*	1.26	10.71	8.61	45.33 bc
2,4-D + 2.5% Bonus-NPK*	1.29	10.60	8.35	43.93 c
2,4-D + 5% Bonus-NPK*	1.40	10.71	7.82	47.51 abc
P-value	*0.1898*	*0.8052*	*0.4212*	*0.0407*

[x] Titratable acidity
[ns] No significant differences
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
*after physiological fruit drop

Table 3.16: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Marisol Clementine' mandarin, Citrusdal 2011/12.

Treatment	External quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	-------(mm.)------		(1 to 6)	(1 to 4)	----------(mm.)----------		
Control	53.79[ns]	53.72[ns]	7[ns]	2[ns]	4.05 a[z]	1.90 c	4.50[ns]
2,4-D*	55.33	55.81	7	2	3.42 b	1.97 bc	6.60
2,4-D + KMAX*	54.67	55.94	7	2	3.95 a	1.95 bc	5.52
2,4-D + Carbology K*	52.97	52.15	7	2	3.60 b	1.82 bc	5.24
2,4-D + Quatro optifruit*	53.62	53.78	7	2	3.40 b	2.16 a	5.99
2,4-D + Bonus NPK*	56.04	55.38	7	2	3.36 b	2.02 bc	7.61
P-value	*0.2256*	*0.1130*	*0.4099*	*0.7890*	*0.0001*	*0.0036*	*0.1212*

[x] Equatorial region
[ns] No significant differences
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
*after physiological fruit drop

Table 3.17: Effect of different combinations of chemical applications on internal fruit quality of 'Marisol Clementine' mandarin fruit at commercial harvest, Citrusdal 2011/12.

Treatment	Internal quality			
	TA[x]	°Brix	°Brix:TA	Juice content (%)
Control	1.23 ab[z]	12.26 [ns]	8.40 [ns]	48.78 [ns]
2,4-D*	1.17 b	10.38	8.97	47.40
2,4-D + KMAX*	1.16 b	9.90	8.70	46.91
2,4-D + Carbology K*	1.23 ab	8.53	8.53	49.75
2,4-D + Quatro optifruit*	1.27 a	8.53	8.54	49.39
2,4-D + Bonus NPK*	1.29 a	7.92	7.92	48.41
P-value	*0.0500*	*0.2218*	*0.2786*	*0.3209*

[x] Titratable acidity
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 3.18: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Mor' mandarin, Paarl 2011/12

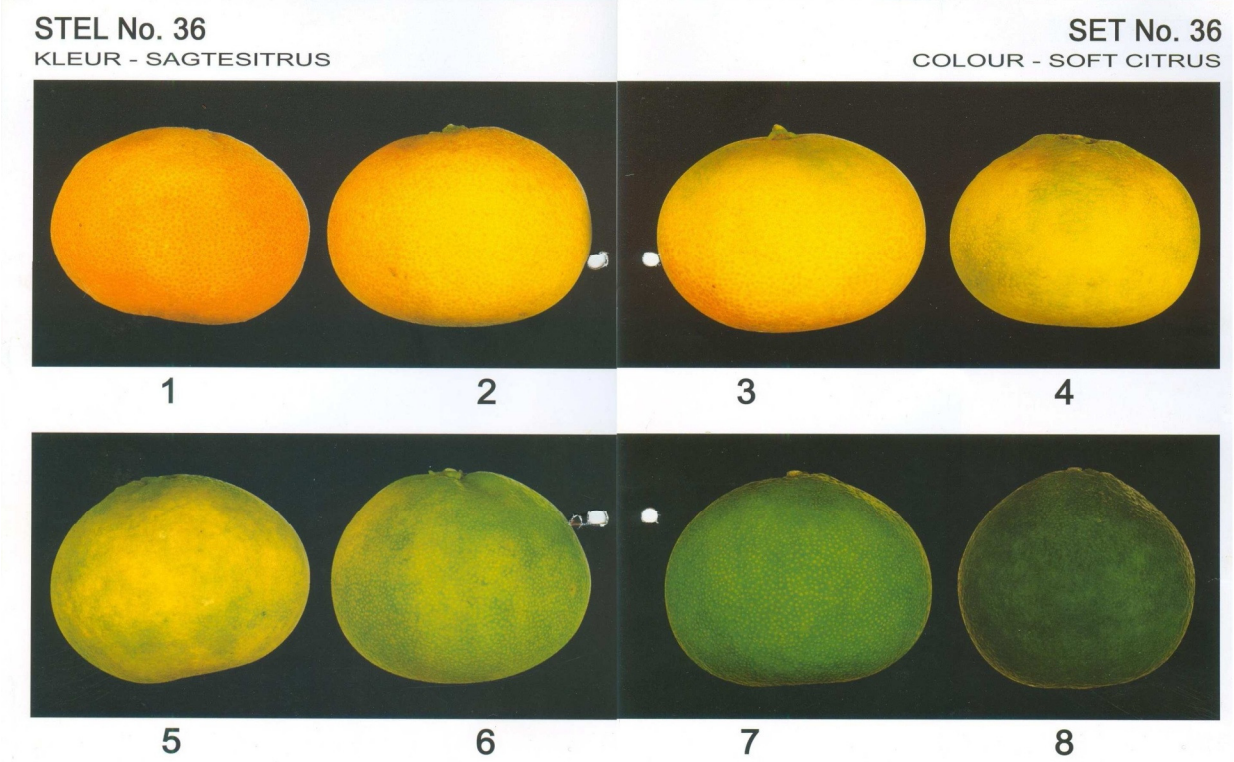
Treatment	External quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	----------(mm.)----------		-(1 to 6)-	--(1 to 4)--	----------(mm.)----------		
Control	61.80dc[z]	48.07b	1 [ns]	1 [ns]	2.41 [ns]	1.63 b	2.29 b
2,4-D*	65.09a	50.43a	1	1	2.61	1.73 b	2.70 a
Bonus NPK*	63.91ab	49.43ab	1	1	2.57	2.53 a	2.38 b
$Ca(NO_3)_2$*	61.60ab	48.46b	1	1	2.47	2.50 a	2.27 b
2,4-D + Bonus NPK*	64.32d	50.30a	1	1	2.59	2.51 a	2.34 b
2,4-D + $Ca(NO_3)_2$*	64.27ab	50.52a	1	1	2.53	2.46 a	2.29 b
2,4-D January	63.17dc	48.66b	1	1	2.59	2.21 ab	2.24 b
2,4-D February	62.02dc	49.02ab	1	1	2.41	1.89 b	2.34 b
P-value	*0.0001*	*0.0060*	*0.0893*	*0.0987*	*0.1436*	*0.0005*	*0.0007*

[x] Equatorial region
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

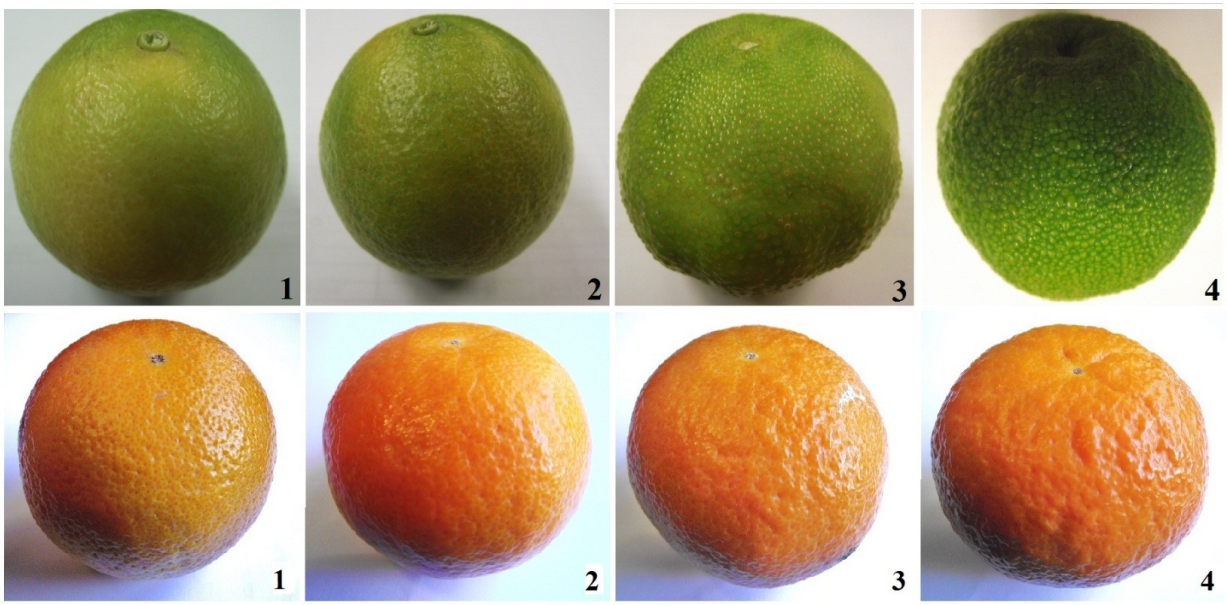
Table 3.19: Effect of different combinations of chemical applications on internal fruit quality of 'Mor' mandarin fruit at commercial harvest, Paarl 2011/12.

Treatment	Internal quality			
	TA[x]	°Brix	°Brix:TA	Juice content (%)
Control	1.33 [ns]	12.32 [ns]	9.27 [ns]	55.06 a[z]
2,4-D*	1.31	12.09	9.25	52.87ab
Bonus NPK*	1.37	12.21	8.95	49.97dc
$Ca(NO_3)_2$*	1.29	12.19	9.54	51.26bc
2,4-D + Bonus NPK*	1.31	11.75	8.97	49.85dc
2,4-D + $Ca(NO_3)_2$*	1.30	11.80	9.09	48.10d
2,4-D January	1.36	12.05	8.90	48.87dc
2,4-D February	1.40	12.25	8.77	48.75d
P-value	*0.1827*	*0.4267*	*0.3443*	*0.0001*

[x] Titratable acidity
[ns] No significant differences
[z] Means with a different letter differ significantly at the 5% level (LSD)
*after physiological fruit drop



Appendix 1a: Colour chart for soft citrus (1: Orange, 8: Green) (CRI, 2004)



Appendix 2: Rind coarseness (1: smooth, 4: very coarse) of 'Marisol Clementine' mandarin (top) & 'Mor' and 'Orri' mandarin (bottom).

4. PAPER 2: FOLIAR 2,4-DICHLOROPHENOXY ACETIC ACID (2,4-D) APPLICATION IN JANUARY REDUCES FRUIT SPLITTING AND FRUIT GROWTH RATE OF VALENCIA ORANGE

Abstract

Fruit splitting is a physiological disorder that involves the cracking of the rind at the stylar- and/or navel-end of a fruit and eventual splitting and premature drop of affected fruit. 'Navel' and 'Valencia' orange are particularly susceptible due to the presence of the navel structure in 'Navel' orange and thin rind of 'Valencia' orange. Foliar applications of different combinations of 2,4-dichlorophenoxy acetic acid (2,4-D), gibberellic acid (GA_3) and mineral nutrients calcium (Ca) and potassium (K) to reduce the disorder in split-prone orange cultivars, were evaluated over two consecutive seasons, 2010/2011 and 2011/2012. Studies were carried out on two different orange cultivars, viz., Bahianina Navel and Midknight Valencia, at two different localities, viz., Citrusdal and Clanwilliam. Treatments were applied directly after physiological fruit drop (November/December) and at two later dates in summer (January and February). January application of 10 $mg \cdot L^{-1}$ 2,4-D reduced splitting in 'Midknight Valencia' orange. Post physiological fruit drop application of 10 $mg \cdot L^{-1}$ 2,4-D had no effect on rind coarseness and no styles remained attach. Except for a reduction in juice content (%) and total acidity (TA), there were no effects on the °Brix, and °Brix:TA at commercial harvest. All the 2,4-D treatments reduced the growth rate of fruit, resulting in smaller fruit at time of harvest. Further research should be conducted in order to validate the foliar application of 2,4-D, later than blossom period on orange cultivars.

Additional keywords: *Citrus, physiological disorder, physiological fruit drop, rind coarseness, synthetic auxin, calcium, potassium*

4.1. Introduction

Citrus fruit splitting is a physiological disorder caused by pressure resulting from the expanding pulp of an individual fruit on the rind (Almela et al., 1994; Barry and Bower 1997; Bower et al., 1992). Rind of affected fruit eventually crack and fruit split open, leading to fruit loss. Factors contributing to weak rind are also responsible for increased susceptibility of fruit to splitting and include insufficient nutrition, specifically of calcium (Ca) and potassium (K) (Bar-Akiva, 1975; De Cicco et al., 1988; Erickson, 1957), warm and humid climatic conditions (Almela et al., 1994; Barry and Bower, 1997; Rabe et al., 1990), irregular water supply (De Cicco et al., 1988) and heavy crop load (Barry and Bower 1997; Rabe et al., 1990). Mandarin and mandarin hybrids, as well as 'Valencia' orange fruit generally develops fruit with thin rind and are therefore particularly susceptibility to split.

However, 'Navel' orange cultivars also experience splitting due to the development of a secondary fruitlet (navel) at the stylar-end of the primary fruit (García-Luis et al., 1994; Rabe and Van Rensburg, 1996). These structures hamper the structural integrity of the rind (García-Luis et al., 1994) and as fruit start to grow, the rind of the primary fruit cracks and split open as the navel starts to protrude. Fruit with large navels are therefore more prone to splitting, as was found with 'Washington navel' orange (García-Luis et al., 1994; Lima et al., 1980; Wager, 1939).

Bloom application of the synthetic auxin 2,4-D increased the percentage closed navels (by up to 42%) in six different navel cultivars and reduced the size of the navel-end (by up to 5 mm.) (Mupambi, 2010). This effect has various benefits, *viz.* a decrease in susceptibility to insect pests such as False Codling moth (Thaumatotibia (Cryptophlebia) leucotetra, navel-end rot, and more importantly, a reduction in the fruit's suceptibilty to splitting. Kassem et al. (2011) on 'Washington' navel, reported a significant increase in fruit size with a treatment of 2,4-D at 20 $mg{\cdot}L^{-1}$ in combination with calcium chloride (0.5%) applied at fruit diameter of 15 mm.

In 'Valencia' orange, splitting was reduced and fruit size increased by a treatment of 2,4-D at 10 or 20 $mg{\cdot}L^{-1}$ and KCl (150 kg·ha) at the end of physiological fruit drop (Borroto et al., 1981). Erner et al. (1993) reported an increase in fruit size of 'Shamouti' and 'Valencia' oranges with the application of 20 ppm 2,4-D and 5% Bonus-NPK 6 to 8 weeks after full bloom.

The aim of this study is to evaluate the effect of 2,4-D treatments later than physiological fruit drop, on fruit splitting, –size and important internal and external quality parameters of 'Navel' and 'Valencia' orange. It is hypothesised that foliar application of 10 $mg{\cdot}L^{-1}$ 2,4-D after physiological fruit drop, either alone or in combination with GA_3, Ca or K, may reduce the severity of fruit splitting and increase fruit size of 'Navel' and 'Valencia' orange.

4.2. Materials and Methods

4.2.1. Plant material and treatments

'Bahianina Navel' orange, Clanwilliam

The experiment was conducted in Clanwilliam (32°33S 18°83'E), South Africa, on 'Bahianina Navel' orange trees on 'Rough' lemon rootstock. The orchard was planted in 2000, with a spacing of 5.5 m between rows, 3.5 m between trees in a North-South row-direction. Treatments were applied as foliar applications directly after physiological fruit drop (6 December 2010) when the average fruitlet diameter was 27 mm, as well as at two later dates, 13 January 2011 (±50 mm) and 14 February 2011 (±61 mm) as summarised in Table 4.1.

'Midknight Valencia' orange, Citrusdal

2010/11. The experiment was conducted in Citrusdal (32°49'S 18°97'E), South Africa, on 'Midknight Valencia' orange trees, grafted on 'Rough' lemon [(*Citrus jambhiri* (Lush)] rootstock. The orchard was planted in 1982, at a spacing of 6.4 m between rows, 2.25 m between trees in a North-South row direction. Treatments were applied as foliar applications directly after physiological fruit drop (6 December 2010) when the average fruitlet diameter was 22 mm, as well as at two later dates, 13 January 2011 (±40 mm) and 14 February 2011 (±48 mm) as summarised in Table 4.1.

2011/12. The same orchard and trees were used for the experiment as in 2010/11 and the same treatments as in 2010/2011were applied after physiological fruit drop (1

December 2011) at an average fruitlet diameter of 21 mm and on 14 January 2012 (±38 mm) and 18 February 2012 (±46 mm).

4.2.2. Spray material and application method

The iso-octyl ester formulation of 2,4-D (10 $mg.L^{-1}$) (Dow AgroSciences (Pty.) Ltd.) was used on both cultivars and applied as treatments in December, January and February. On 'Midknight Valencia', 5% Bonus-NPK (Haifa Chemicals Ltd) was applied both on its own and in a tank-mix with 2,4-D, while 2% $Ca(NO_3)_2$ (Omnia Nutriology®) was applied as tank-mix with 2,4-D and 20 $mg.L^{-1}$ Gibberellic acid (GA_3) (ProGibb®, Philagro SA (Pty) Ltd) was applied on its own. A non-ionic wetting agent (Break-Thru®, Villa Crop Protection) with the active ingredient polyether-polymethylsiloxanecopolymer (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 treatments that included 2,4-D and GA_3. Applications were made using a hand gun sprayer until runoff, at approximately 10 L per tree. For all the control treatments, trees were sprayed directly after physiological fruit drop with water and the non-ionic wetting agent. The same treatments and method of application were used on 'Midknight Valencia' in 2011/12 as in 2010/11. In 2011/12, 'Bahianina Navel' was excluded from the experiment, as results from 2010/11 showed a negative effect of all the treatments on fruit size, as well as no effect on average total number split fruit per tree.

4.2.3. Experimental Design

Each experiment was carried out in a commercial orchard where standard production practices were utilised. In all the experiments, each treatment was repeated as ten single tree replicates in a randomized complete block design with buffer trees between treated trees as well as buffer rows. Only healthy uniform trees were used.

4.2.4. Data collection and evaluations

Orchards were monitored for initiation of fruit splitting and split fruit were removed and counted at two- to three-weekly intervals from initiation of split, until harvest. Splitting is expressed as the total number split fruit removed and counted per tree and not as the percentage of the total fruit, as the total yield per tree was not determined in the experiment. In all the experiments, 10 fruit were randomly selected and tagged on 2,4-D replicates after chemical treatments was applied. The diameters (mm.) of the tagged fruit were measured at monthly intervals until harvest. At harvest the growth rate ($mm.day^{-1}$) of each fruit were calculated by subtracting the initial diameter from the final diameter and dividing it by the number of days from initial measurement until final measurement. At commercial harvest, 12 fruit were sampled per tree, 6 from each row-side, to evaluate the treatment effect on important parameters of internal and external fruit quality as described below.

For external qualities, fruit rind colour was determined using the CRI colour chart for oranges no. 34 (Citrus Research International (CRI), 2004; Appendix 1a) with 8 being dark green and 1 a 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). An

electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) was used to measure fruit diameter, fruit length and pedicel diameter (mm.).

Fruit were cut into half along the longitudinal plane for internal quality determinations. An electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) was used to measure rind thickness for each fruit at the equatorial region and stylar-end as described in Paper 1. The fruit were then juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth and the juice content (%) was determined by dividing the weight of the juice by the total fruit weight. °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 °Brix:TA ratio was calculated by dividing the °Brix values by the TA values.

4.2.5. Statistical analysis

Statistical analyses of variance (ANOVA) were performed using PROC GLM of the SAS Programme (version 9.1, SAS Institute Inc., Cary, NC, USA). Mean separation was done by the least significant difference (LSD) test where applicable ($P = 0.05$).

4.3. Results

4.3.1. 2010/11

'Bahianina Navel' orange, Clanwilliam

Fruit splitting: There was no occurrence of fruit splitting in this particular orchard in 2011 and treatment effect on fruit splitting could therefore not be evaluated (Table 4.2).

Fruit size: All the treatments significantly reduced the growth rate ($mm.day^{-1}$) in comparison to the control (Table 4.3).

Fruit quality: There were no significant differences in fruit dimensions, rind colour and –coarseness between any of the treatments and the control. There were no significant differences in rind thickness or pedicel diameter (Table 4.4). There were no significant effect of treatments on the TA, °Brix, °Brix:TA or juice content (%) (Table 4.5).

'Midknight Valencia' orange, Citrusdal

Fruit splitting: Although there were no significant differences between any of the treatments or the control, 2,4-D treatments in January and February were most successful and reduced splitting most effectively (Table 4.2).

Fruit size: All the treatments significantly reduced the growth rate (mm/day) in comparison to the control (Table 4.3).

Fruit quality: None of the treatments had a significant effect on fruit dimensions (diameter and length), rind colour and –coarseness (Table 4.6). There was also no significant difference in rind thickness at either the stylar-end or equatorial region of the fruit. Pedicel diameter was increased by the 2,4-D treatment after physiological fruit drop, and in February, as well as by the $Ca(NO_3)_2$ treatment (Table 4.6). The TA of fruit from the 2,4-D treatments in January and February, were significantly lower compared to the control fruit (Table 4.7). In contrast, there was no significant difference in °Brix induced by any treatments, while the °Brix:TA ratio of fruit treated with 2,4-D in January and February were significantly higher than that of control fruit. Treatment of 2,4-D alone, after physiological fruit drop and in January as well as applied in a tank-mix with Bonus-NPK, as well as the GA_3 treatment in January, significantly reduced the juice content (%) of fruit, compared to the control (Table 4.7).

4.3.2. 2011/12

'Midknight Valencia' orange, Citrusdal

Fruit splitting: Fruit splitting was more severe in the 2011/12 production season, compared to the 2010/11. The total number of split fruit was reduced by all the treatments, with the January application of 2,4-D and GA_3, as well as the tank-mix application of 2,4-D and $Ca(NO_3)_2$ the most successful (Table 4.8).

Fruit size: All the 2,4-D treatments reduced the fruit growth rate compared to the control, with the December application at physiological fruit drop being the most severe (Table 4.9).

Fruit quality: Treatments had no effect on fruit dimensions (diameter and length), rind thickness, as well as rind colour and -coarseness. Pedicel diameter was increased by the 2,4-D treatment applied directly after physiological fruit drop, as well as when applied in February, and by the 2.4-D and $Ca(NO_3)_2$ treatment (Table 4.10). January and February 2,4-D applications reduced the titratable acidity (TA), resulting in a non-significant increase in the °Brix:TA ratio (Table 4.11). Both 2,4-D applied alone as well as in combined with Ca and K after physiological fruit drop, as well as the 2,4-D and GA_3 treatment in January, significantly reduced the juice content (%) of fruit, compared to control (Table 4.11).

4.4. Discussion

The foliar application of 10 $mg \cdot L^{-1}$ 2,4-D in January reduced fruit splitting most effectively in 'Valencia' orange in both seasons, thereby concurring with Borroto et al., (1981). Treatment consisting of 2.4-D and $Ca(NO_3)_2$ also successfully reduced fruit splitting in 'Midknight Valencia'. Application of 2,4-D directly after physiological fruit drop (December) and in February were least successful and failed in reducing the disorder significantly in comparison to the control. There was no fruit splitting occurrence in 'Bahianina Navel' orange and treatment effect on fruit splitting of this cultivar could therefore not be evaluated. The bloom application of 2,4-D increased the percentage closed navels (by up to 42%) in six different navel cultivars and reduced the size of the navel-end (by up to 5 mm.) (Mupambi, 2010). Reduction in the size of the navel-end reduces the susceptibility of fruit to splitting (García-Luis et al., 1994) and bloom application of 2,4-D are therefore an effective method of controlling fruit splitting of Navel cultivars.

There was no significant effect of treatments on rind colour, -coarseness and thickness, but the average pedicel diameter of fruit treated with 2,4-D was significantly increased. Mesejo et al. (2003) reported an increase in the pedicel diameter resulting from an increase in the central xylem cylinder as well as the number and the average diameter of xylem vessels, after foliar application of 15 $mg \cdot L^{-1}$ 2,4-D after physiological fruit drop. With an increase in pedicel diameter, an increase in transport effectiveness of water, nutrients and assimilates is established by the increase in the capacity of the vascular system that connects the source (leaves) to the sink (developing fruit) (Bustan et al., 1995). Therefore, with an increase in pedicel diameter, bigger fruit is expected. However, all the 2,4-D treatments as reported here, resulted in a significant reduction in the growth rate of treated fruit and significantly smaller fruit at time of harvest. This is of particular concern in 'Midknight Valencia', as small fruit size is already a problem in this cultivar and concur with results of Mupambi (2010) who reported a reduction in fruit size of 'Marisol Clementine' mandarin with the bloom application of 2,4-D. However these results are contradictory to previous results where 2,4-D increased size of 'Navel' and 'Valencia' orange (Erner et al., 1993; Kassem et al., 2011).

Except for reduced juice content (%) and TA, there was generally no significant effect of the treatments on the °Brix, as well as °Brix:TA ratio. Although fruit splitting of 'Midknight Valencia' was reduced by a medium cover foliar application of 10 $mg \cdot L^{-1}$ 2,4-D in January, the treatment result in a reduced growth rate of treated fruit resulting in smaller fruit at time of harvest. Treatment with 2,4-D also resulted in a reduction in the juice content (%) of fruit and the treatment of orange cultivars with 2,4-D after physiological fruit drop could therefore not be recommended. Bloom

application seems to be the optimum timing of 2,4-D treatments on 'Navel' orange and similar concentration and timing of 2,4-D should also be evaluated on 'Valencia' cultivars.

4.5. References

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Table 4.1: Summary of foliar chemical applications indicating concentrations, timing, cultivars and experimental site.

Treatments	Average fruitlet diameter at time of foliar application (mm)		
	'Midknight Valencia', Citrusdal		'Bahianina Navel', Clanwilliam
Year	**2010/11**	**2011/12**	**2010/11**
5% Bonus-NPK*	22	21	-
2% $Ca(NO_3)_2$ + 2,4-D*	22	21	-
20 mg·L^{-1} GA_3 January	40	38	-
10 mg·L^{-1} 2,4-D*	22	21	27
2,4-D January	40	38	50
2,4-D February	48	46	61
2,4-D and 5% Bonus-NPK*	22	21	-

For detail on chemicals see section 4.2.2
*Applied directly after physiological fruit drop

Table 4.2: Effect of different combinations of chemical applications on total fruit splitting of various orange cultivars and sites in the production season of 2010/11.

Treatment	Ave. nr. split fruit per tree	
	'Midknight Valencia', Citrusdal	**'Bahianina Navel', Clanwilliam**
Control	25[ns]	0
5% Bonus-NPK*	17	0
2,4-D + 2% $Ca(NO_3)_2$*	19	0
20 mg·L^{-1} GA_3 January	25	0
10 mg·L^{-1} 2,4-D*	25	0
2,4-D January	13	0
2,4-D February	18	0
2,4-D and 5% Bonus-NPK*	28	0
P-value	*0.0839*	

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

[ns] No significant differences

*after physiological fruit drop

Table 4.3: Effect of treatments on fruit growth rate (mm.day^{-1})of various orange cultivars in the production season of 2010/11.

Treatment	Ave. fruit growth rate (mm/day)	
	'Midknight Valencia', Citrusdal	**'Bahianina Navel', Clanwilliam**
Control	0.17a[z]	0.30a
10 mg·L^{-1} 2,4-D*	0.12c	0.29b
2,4-D January	0.14b	0.28b
2,4-D February	0.14b	0.29b
P-value	*0.0021*	*0.0173*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

*after physiological fruit drop

Table 4.4: The influence of different timings of 2,4-D (10 mg·L^{-1}) foliar applications on external fruit quality parameters of 'Bahianina Navel' orange in Clanwilliam 2010/11.

Treatment	External fruit qualities						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R^{x})**
	-------(mm.)------		(1 to 6)	--(1 to 4)--	----------(mm.)----------		
Control	82.84^{ns}	78.77^{ns}	2^{ns}	3^{ns}	3.96^{ns}	7.87^{ns}	8.23^{ns}
2,4-D*	80.68	76.66	2	3	4.04	7.81	8.02
2,4-D January	79.83	74.85	2	3	3.86	7.49	7.46
2,4-D February	83.80	79.00	2	3	4.08	8.06	8.09
P-value	*0.0705*	*0.0619*	*0.1773*	*0.1340*	*0.0605*	*0.0768*	*0.2500*

[x] Equatorial region
[ns] No significant differences
*after physiological fruit drop

Table 4.5: The influence of different timings of 2,4-D (10 mg·L^{-1}) foliar applications on internal fruit quality parameters of 'Bahianina Navel' orange, Clainwilliam in 2010/11.

	Internal qualities			
Treatment	**TA[x]**	**°Brix**	**°Brix:TA**	**Juice content (%)**
Control	1.23 [ns]	9.19 [ns]	7.50[ns]	42.60 [ns]
2,4-D*	1.23	9.17	7.52	40.72
2,4-D January	1.15	9.12	7.35	41.36
2,4-D February	1.21	9.18	7.66	40.13
P-value	*0.0589*	*0.3697*	*0.0721*	*0.0601*

[x] Titratable acidity
[ns] No significant differences
*after physiological fruit drop

Table 4.6: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Midknight Valencia' orange, Citrusdal 2010/11.

Treatment	External quality						
	Diameter	**Height**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	----------(mm.)----------		-(1 to 6)-	--(1 to 4)--	----------(mm.)----------		
Control	71.57 [ns]	75.42 [ns]	1 [ns]	3[ns]	3.53 c[z]	5.34 [ns]	8.71 [ns]
2,4-D*	72.84	76.82	2	3	4.41 a	5.20	8.70
Bonus NPK*	71.48	75.39	2	3	3.65 c	5.03	8.57
2,4-D + Ca $(NO_3)_2$*	71.94	74.19	2	3	4.41 a	5.19	8.69
2,4-D + Bonus NPK*	71.77	76.70	2	3	3.84 bc	5.23	9.32
GA_3 January	71.02	75.28	2	3	3.38 c	3.72	6.75
2,4-D January	70.62	74.33	2	3	3.75 c	5.20	8.46
2,4-D February	71.07	75.07	2	3	4.21 a	5.42	8.44
P-value	*0.6455*	*0.0839*	*0.1525*	*0.0872*	*0.0001*	*0.0680*	*0.1508*

[x] Equatorial region
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 4.7: Effect of different combinations of chemical applications on internal fruit quality of 'Midknight Valencia' orange, Citrusdal 2010/11.

Treatment	Internal quality			
	TA[x]	**°Brix**	**°Brix:TA**	**Juice content (%)**
Control	1.27 ab[z]	9.84[ns]	7.78 cd	48.90a
2,4-D*	1.32 a	9.74	7.43 d	46.17b
Bonus NPK*	1.30 a	9.75	7.50 d	47.38ab
Ca $(NO_3)_2$*	1.31 a	10.17	7.79 cd	48.09a
2,4-D + Bonus NPK*	1.26 bc	9.96	8.17 bc	43.20c
GA_3 January	1.22 bc	9.82	8.07 bc	43.68c
2,4-D January	1.16 cd	9.88	8.53 ab	43.52c
2,4-D February	1.10 d	9.59	8.72 a	48.44a
P-value	*0.0001*	*0.3698*	*0.0001*	*0.0001*

[x] Titratable acidity
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 4.8: Effect of different combinations of chemical applications on total fruit splitting of various orange cultivars in the production season of 2011/12.

Treatment	Ave. nr. split fruit per tree	
	'Midknight Valencia', Citrusdal	**'Bahianina Navel', Clanwilliam**
Control	52a[z]	-
5% Bonus-NPK*	28bc	-
2% $Ca(NO_3)_2$ + 2,4-D*	26c	-
20 mg·L^{-1} GA_3 January	25c	-
10 mg·L^{-1} 2,4-D*	40b	-
2,4-D January	28bc	-
2,4-D February	38b	-
2,4-D and 5% Bonus-NPK*	34bc	-
P-value	*0.0006*	

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

*after physiological fruit drop

Table 4.9: Effect of treatments on fruit growth rate of 'Midknight Valencia' orange in Citrusdal 2011/12.

Treatment	Fruit growth rate ($mm.day^{-1}$)
	'Midknight Valencia', Citrusdal
Control	0.17a[z]
10 $mg \cdot L^{-1}$ 2,4-D*	0.12c
2,4-D January	0.14b
2,4-D February	0.14b
P-value	*0.0021*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)

*after physiological fruit drop

Table 4.10: Effect of different combinations of chemical applications on external fruit quality at harvest of 'Midknight Valencia' orange, Citrusdal 2011/12.

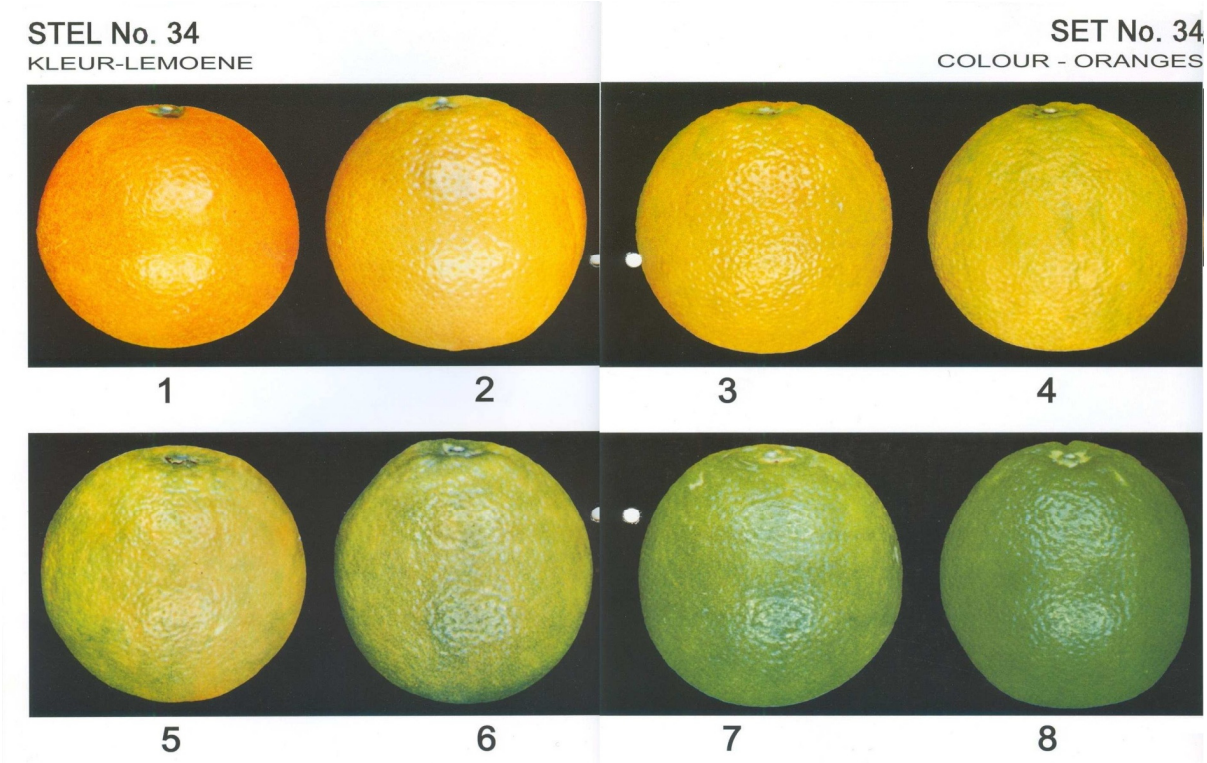
Treatment	External quality						
	Diameter	**Length**	**Rind colour**	**Rind coarseness**	**Pedicel diameter**	**Rind thickness (stylar-end)**	**Rind thickness (E-R[x])**
	----------(mm.)----------		-(1 to 6)-	--(1 to 4)--	----------(mm.)----------		
Control	69.54 [ns]	74.12 [ns]	2 [ns]	3 [ns]	3.67 c [z]	4.84 [ns]	7.75 [ns]
2,4-D*	70.84	75.33	2	3	4.52 a	5.14	7.71
Bonus NPK*	71.48	73.39	2	3	3.59 c	4.97	8.02
Ca $(NO_3)_2$*	71.77	73.45	2	3	4.21 a	5.04	8.14
2,4-D + Bonus NPK*	70.45	75.92	2	3	3.84 bc	5.13	8.32
GA_3 January	71.11	75.28	2	3	3.51 c	4.72	7.04
2,4-D January	70.14	74.27	2	3	3.70 c	5.11	8.46
2,4-D February	70.07	75.19	2	3	4.41 a	4.42	8.09
P-value	*0.6455*	*0.0839*	*0.1525*	*0.0987*	*0.0001*	*0.0680*	*0.1508*

[x] Equatorial region
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 4.11: Effect of different combinations of chemical applications on internal fruit quality of 'Midknight Valencia' orange, Citrusdal 2011/12.

Treatment	Internal quality			
	TA	**°Brix**	**°Brix:TA**	**Juice content (%)**
Control	1.37ab[z]	9.61[ns]	7.43 cd	49.01 a
2,4-D*	1.42 a	9.85	7.41 d	46.24b
Bonus NPK*	1.40 a	9.86	7.52 d	47.49ab
Ca $(NO_3)_2$*	1.43 a	10.28	7.81 cd	48.12a
2,4-D + Bonus NPK*	1.35 bc	10.06	8.21 bc	44.69c
GA_3 January	1.31 bc	9.94	8.19 bc	43.91c
2,4-D January	1.24 cd	9.97	8.64 ab	43.18c
2,4-D February	1.21 d	9.70	8.84 a	48.99a
P-value	*0.0001*	*0.3698*	*0.0001*	*0.0001*

[x] Titratable acidity
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop



Appendix 1: Colour chart for oranges (1: Orange, 8: Green) (CRI, 2004).

Appendix 2: Rind coarseness of oranges (1: smooth, 4: very coarse).

5. PAPER 3: FOLIAR 2,4-DICHLOROPHENOXY ACETIC ACID (2,4-D) AND BONUS-NPK APPLICATION REDUCES FRUIT SPLITTING IN 'MARISOL CLEMENTINE' MANDARIN (*Citrus reticulata* Blanco) BY INCREASING RIND THICKNESS, STRENGTH AND FRUIT DIAMETER

Abstract

'Marisol Clementine' mandarin (*Citrus reticulata* Blanco) fruit is prone to splitting, a physiological disorder which entails cracking of the rind at the stylar-end and eventual splitting and abscission of the fruit. Foliar application of the synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D), has the potential to reduce the disorder in mandarin cultivars, although the method of action is unknown. The effect of foliar applications of 2,4-D on its own, or in combination with potassium (K) on fruit splitting and fruit anatomy of 'Marisol Clementine', was evaluated in an orchard prone to the disorder in Paarl, South Africa. Treatments consisted of 10 mg·L^{-1} 2,4-D and a tank-mix of 10 mg·L^{-1} 2,4-D + 5% Bonus-NPK, applied after the physiological fruit drop period. Fruit were sampled prior to visual split initiation, at the start of visual split initiation, one month after visual split initiation and at commercial harvest and the treatment effect on fruit anatomy determined. Both treatments reduced fruit splitting significantly, however internal quality was unaffected. Treatments increased rind thickness and rind strength throughout fruit development. Both fruit diameter and –length increased to such an extent that the fruit shape was altered (reduced d/l-ratio). Rind coarseness of treated fruit was also significantly increased, reducing cosmetic appearance.

Additional Keywords: *citrus, synthetic auxin, rind coarseness, stylar-end splitting*

5.1. Introduction

Citrus fruit splitting, a physiological disorder of the fruit rind occurs in cultivars of the thin-rind mandarin (*Citrus reticulata* Blanco) and mandarin hybrids (Almela et al., 1994; Rabe and Van Rensburg, 1996) as well as 'Navel' (De Cicco et al., 1988) and 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] (Borroto et al., 1981).

The rind of a citrus fruit is comprised of two distinctive layers: a spongy, white internal layer, the albedo (mesocarp) and an external layer, the flavedo (exocarp) (Monselise, 1986). The albedo contains vascular bundles, has large inter-cellular spaces and is in immediate contact with the inner, edible segments of the fruit. The flavedo surrounds both the pulp and the albedo of the fruit and is responsible for the colourful external appearance of the fruit (Monselise, 1986). During stage I of citrus fruit development (cell division), the majority of the rind cells (flavedo and albedo) are formed. During stage II (cell growth), cell division of the rind declines and cells of the pulp starts to expand (Bain, 1958). The pressure applied by the rapidly expanding pulp, leads to the initiation of fruit splitting at the stylar- or navel-end of the fruit, the area where the rind is the thinnest and/or structurally weaker than other areas of the rind (Coit, 1915). Split fruit abscise from the tree which leads to a reduction in yield, and requires additional labour to sanitise affected orchards.

Foliar applications of synthetic auxins such as 2,4-dichlorophenoxy propionic acid (2,4-DP) and 3,5,6-trichloro-2-piridil oxyacetic acid (3,5,6-TPA), to increase fruit size, is a

common cultural practice in citrus orchards (Agusti et al., 2002). Fruit diameter is positively related to rind thickness (Morgan et al., 2005) and as a result, these foliar applied synthetic auxin sprays, also result in increased rind thickness and coarseness (Coggins and Hield, 1968), mainly due to elongation of oil glands in the flavedo (Stewart and Klotz, 1947). This effect of the synthetic auxin, 2,4-dichlrophenoxy acetic acid (2,4-D), has subsequently been shown to reduce fruit splitting in citrus cultivars of Nova mandarin as well as various Valencia cultivars (Almela et al., 1994; Borroto et al., 1981; García-Luis et al., 2001). Reduction of fruit splitting in 'Marisol Clementine' by foliar application of 2,4-D during full bloom, was accompanied by increased rind coarseness and attached styles at harvest, reducing cosmetic appearance of treated fruit (Mupambi, 2010).

Potassium (K) deficiency in citrus trees results in small fruit with thin and smooth rinds (Chapman, 1968) and an increased susceptibility to splitting (Koo, 1961; Morgan et al., 2005). Supplementing citrus trees with K increases both leaf and rind K concentration, resulting in bigger fruit with thicker rind, which have been shown to reduce fruit split occurrence (Bar-Akiva, 1975; De Cicco et al., 1988). Combining K with auxins improved its effect, both as fruit size enhancer in 'Shamouti' and 'Valencia' orange (Erner et al., 1993) and in reducing fruit splitting of 'Nova' mandarin (Greenberg et al., 2006). Greenberg et al., (2006) reported a reduction in splitting of 'Nova' mandarin, as well as increase in fruit diameter, resulting from foliar application of 40 $mg \cdot L^{-1}$ 2,4-D + Bonus-NPK (Haifa Chemicals Ltd.) applied after physiological fruit drop. Except for an increase in rind thickness, a reduction of splitting by foliar applications of 2,4-D, was also due to

an increase in rind resistance to puncturing (Almela et al., 1994). In addition, 2,4-D altered the fruit dimensions positively, relieving the tension at the stylar-end of the fruit and reducing the susceptibility of the fruit to splitting (García-Luis et al., 2001).

The objective of this study was to determine the effect of foliar applications of the synthetic auxin 2,4-D, both on its own and in combination with K on aspects relating to the fruit rind and shape as well as the internal quality of split-prone mandarin cultivar 'Marisol Clementine'.

5.2. Materials and Methods

5.2.1. Plant material and treatments

The experiment was conducted in Paarl (33°69'S 18°95'E), South Africa on 'Marisol Clementine' mandarin trees on 'Carrizo citrange' (*C.sinensis* [L.] Osbeck × *P. trifoliata* [L.] Raf.) rootstock. The orchard was planted in 1994, at a spacing of 4.8 m between rows, 2 m between trees and a North-South row direction.

5.2.2. Experimental Design

The experiment was carried out in a commercial orchard where standard production practices were utilised. Each treatment consisted of five single tree replicates in a randomized complete block design with buffer trees between treated trees as well as buffer rows. Only healthy uniform trees were used.

5.2.3. Spray material and application method

A concentration of 10 mg·L^{-1} of the dimethyl amine salt of 2,4-D (Dow AgroSciences (Pty.) Ltd.) (480 g.l^{-1} active 2,4-D as phenoxy compound) was used both on its own and in a tank-mix solution with 5% Bonus-NPK (Haifa Chemicals Ltd.) (38.5% active K). Foliar applications were made after physiological fruit drop (30 November 2011) when average fruitlet diameter was 13 mm. A non-ionic wetting agent (Break-Thru®, Villa Crop Protection) with the active ingredient polyether-polymethylsiloxanecopolymer (1000 mg·L^{-1}) was added to the spray solution at a rate of 5 ml per 100 L of spray solution in all the treatments that included 2,4-D. Applications were made using a hand gun sprayer until run-off, at approximately 10 L per tree. For the control treatment, trees were sprayed on the same day with water containing the non-ionic wetting agent.

5.2.4. Data collection and evaluations

During 2012, trees were monitored on 9 January (prior to visual split initiation), 8 February (start of visual split initiation) and 6 March (one month after visual split initiation) for fruit split initiation and all split fruit were removed and counted. Splitting is expressed as the total number split fruit removed and counted per tree and not as the percentage of the total fruit, as the total yield per tree was not determined in the experiment. In addition, a 5-fruit sample was randomly selected from each replicate, at these three different dates and rind anatomy evaluated as described below.

In order to measure rind cutting force during split initiation the following procedure was used. Two 10 × 10 mm pieces of the rind were removed from opposite cheeks at the equatorial region and one from the stylar-end of the 5 fruit per replicate. The pieces were then placed on a Texture Analyser (TAXT2i, Stable Microsystems, England) HDP/BSK blade set. A blade set knife of the Texture Analyzer, attached to the probe carrier was used to cut the rind pieces. The load cell was calibrated to 250 N and the cutting was done at a speed of 1 $mm.s^{-1}$. The average peak cutting force values (N) of five fruit per replication were measured for both the stylar-end and equatorial region of the fruit. In order to visually see the first sign of splitting, a dissection microscope (Carl Zeis ERc5s) was used to photograph control and 2,4-D + Bonus-NPK-treated fruit on 17 January (visual initiation of split) and of split fruit in March (1 month before harvest).

In addition, 10 fruit per tree were collected at commercial harvest (4 April), to evaluate the treatment effect on fruit shape, rind thickness and –coarseness and internal fruit quality. An electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) was used to measure fruit diameter and length. The diameter to length ratio (d/l-ratio) was determined by dividing the diameter by the length value. Rind coarseness was scored on a scale from 1 to 4 [1 being a smooth rind and 4 a coarse rind (Appendix 2, Paper 1)]. Fruit were then cut into half and the rind thickness of each fruit measured at the equatorial region, as well as at the stylar-end of the fruit, using an electronic calliper.

Internal fruit quality was determined by cutting fruit into half along the longitudinal plane. Fruit were 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. °Brix of 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 °Brix:TA- ratio was calculated by dividing the °Brix values by the TA values.

5.2.5. Statistical analysis

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

5.3. Results

5.3.1. Fruit splitting

Split fruit were first visible with the naked eye from the start of February (Fig. 5.1), however micro-cracks were already visible at the stylar-end scar of sampled control fruit with the dissection microscope on 17 January (Fig. 5.2 A-B). The stylar-end of 2,4-D + Bonus-NPK-treated fruit sampled on 17 January were intact, without micro-cracks and slightly elevated, possibly due to an increased cell number in the rind (Fig. 5.2 C-D). Although the total number of split fruit was reduced by both applications at harvest, treatment with 2,4-D + Bonus-NPK, significantly reduced splitting at evaluation in

February and March, compared to control trees and trees treated with 2,4-D alone (Fig. 5.1).

5.3.2. Rind strength

Application of 10 mg·L^{-1} 2,4-D alone and in combination with 5% Bonus-NPK, increased the rind strength at the stylar-end and in the equatorial region of treated fruit sampled on 8 February and 6 March (Table 5.1, Fig. 5.3). At the first evaluation date (9 January), the rind at the stylar-end of fruit sampled from trees treated with a single 2,4-D application required a significantly higher force (N) to cut, compared to fruit from the control, but no difference was observed in the equatorial region of the fruit. On the second evaluation date (8 February) a higher cutting force was generally required for all sampled fruit, while the force was lower on the third evaluation date (6 March).

5.3.3. Fruit size

At the first evaluation date (9 January), there were no significant differences in fruit diameter between treatments (Fig. 5.4 A). Thereafter, both treatments resulted in significantly larger fruit diameter in February, March and April (harvest) than the control treatment. The fruit length was significantly lower for both foliar sprays in comparison to the control, on all the evaluation dates (January to April) (Fig. 5.4 B). The ratio (diameter to length), differed significantly between the two treatments and control in February and March, with the control fruit having higher ratio values, indicating that these fruit were “flatter” than the 2,4-D-treated fruit (Fig. 5.4 C).

5.3.4. Rind thickness, coarseness and fruit internal quality

After adjusting for differences in fruit diameter, in all the samples, stylar-end rind thickness was lower compared to the equatorial region throughout fruit development (Table 5.2). Stylar-end rind thickness of 2,4-D + Bonus-NPK treated fruit was significantly thicker than that of control fruit when evaluated in January and March (Table 5.2). For 2,4-D treated trees only fruit evaluated in March had thicker stylar-end rind compared to the control (Table 5.2). The rind thickness at the equatorial region of the fruit was only increased by both treatments when evaluated in March (Table 5.2). However, at both the stylar-end and equatorial region, the rind thickness was consistently higher in treated fruit than the control. Both foliar treatments resulted in a significant increase in rind coarseness from February onward (Table 5.3). Both treatments reduced juice content (%), but not significantly when means were adjusted for differences in fruit diameter. There were no significant effect of treatments on the TA, °Brix, or the ratio °Brix:TA (Table 5.4).

5.4. Discussion

Foliar application of 2,4-D (10 $mg \cdot L^{-1}$), either on its own, or in combination with 5% Bonus-NPK directly after physiological fruit drop, reduced fruit splitting of 'Marisol Clementine'. These treatments also resulted in thicker and stronger rind, and changed fruit shape to a elongated shape. Change in these attributes is thought to be associated with the reduction seen in fruit sensitivity to splitting. This concurs with Greenberg et al., (2006) who reported a reduction in fruit splitting in 'Nova' mandarin with 2,4-D + 5%

Bonus-NPK applications after physiological fruit drop. Pre-harvest treatment with 2,4-D facilitated plant cell expansion (Mitchell, 1961), which may offer an explanation for the thicker rind, as well as the enlarged oil glands in the flavedo (Stewart et al., 1951) of treated fruit.

The increase in rind thickness may have been due to the stimulation of cell expansion by both 2,4-D on its own as well as in combination with Bonus-NPK. This concurs with results on 'Nova' mandarin (García-Luis et al., 2001), but not 'Marisol Clementine' (Mupambi, 2010). In both studies the treatments were applied earlier (full bloom to petal drop), compared to this study, viz. at post physiological fruit drop period. Previous studies did not include the fertilizer Bonus-NPK, as reported here. Potassium plays a key role in growth processes of plant cells. An optimum K-content is critical in maintaining cell osmotic potential and turgor pressure, which is essential for cell growth (Leigh and Wynn Jones, 1984). With decrease in plant K-status or availability, K from the cytoplasm is utilized, resulting in a decline in cell growth (McAfee, 2001). In citriculture, supplementing trees with K, increases both leaf and rind K concentration, resulting in bigger fruit with thicker rind, due to stimulation of rind cell growth (Bar-Akiva, 1975; De Cicco et al., 1988; Koo, 1961). Combining K with synthetic auxin, improves its effect, both as a fruit size enhancer (Erner et al., 1993) as well as to reduce fruit splitting (Greenberg et al., 2006). Fruit susceptibility to splitting is known to decrease as rind thickness increases (De Cicco et al., 1988). The thinner rind at the stylar-end of the 'Marisol Clementine', irrespective of treatment, compared to the equatorial region

throughout fruit development, could partly explain the initiation of splitting at this particular area, rather than at the equatorial region.

The increase in rind strength of the 'Marisol Clementine' fruit due to the auxin treatments could be one of the main factors responsible for the reduction in splitting. Rind strength at the stylar-end of the fruit was increased by both 2,4-D treatments in comparison to the control, ranging from 5-7% in January, 18-20% in February and 25-30% in March and support the result on an increase in the rind strength of 'Nova' mandarin, after 2,4-D application (Almela et al., 1994).

García-Luis et al. (1994) reported that the time of split initiation of 'Nova' mandarin fruit coincided with the time of maximum increase in fruit diameter. The biggest difference in fruit diameter of treated fruit relative to the control was seen between 9 January and 8 February (Fig. 5.4). During stage II of citrus fruit development, sap-flow into the clustered juice vesicles of the pulp initiates expansion and causes an increase in fruitlet growth rate (Bain, 1958). As a result, the already developed rind is forced to absorb the tension created by the underlying expanding pulp. Considine and Brown (1981) showed that the tension on fruit pericarp tissue is exerted at a maximum at the polar regions (stylar- and stem-end) of the fruit, which in addition to a thinner stylar-end rind and style-abscission scar, could lead to a more susceptible rind for the initiation of citrus fruit splitting in this particular area. Application of 2,4-D on its own and in combination with Bonus-NPK, increased fruit length, making fruit more cylindrical and less oblate ("flat"). This altered fruit shape (reduced d/l-ratio) was found throughout the observed fruit

development period in this trial. This effect was also reported by Stewart et al. (1951) and results in a relief in the tension exerted on the stylar-end of the fruit and therefore reduces the susceptibility of 2,4-D treated fruit to splitting.

The successful formation of an abscission layer between the style and stylar-end of the rind is important to prevent stylar-end fruit splitting. Natural auxins in plant tissue such as 3-indoleacetic acid (IAA) primarily regulate abscission by blocking the capacity of ethylene to stimulate the abscission of plant material (Borroto et al., 1981; Goren, 1993). The 2,4-D treatments, after physiological fruit drop as reported here, allowed for successful natural style abscission to occur. Visual evaluation of the stylar-end region of treated fruit revealed the rind to be slightly elevated, thickened and without micro-cracks (Fig. 5.2). The treatment seemed to have stimulated rind growth in this area, extending the floral axis beyond the abscission zone of the style, resulting in a compact, solid tissue after style abscission (Fig. 5.2 C-D). In control fruit, the floral axis did not extend towards the style and after abscission of the style, a cavity was formed instead of solid rind tissue (Fig. 5.2 A-B). This cavity reduces the structural integrity of the rind and according to Considine and Brown (1981), increases the stresses at the poles of the fruit and as a result, its susceptibility to splitting. Apparently the timing of the treatment reduced the number of split fruit by increasing both thickness and strength of the stylar-end area of the rind (no cavity), without the negative impact of prevented stylar abscission.

In addition to the positive horticultural effects, 2,4-D can reduce citrus fruit quality by increasing the rind coarseness (Coggins and Hield, 1968) as well as reducing the juice content of treated fruit (Table 5.4). Increased rind coarseness is thought to be a result of the elongation of the oil glands in the flavedo of the rind (Stewart and Klotz, 1947). Applications of 2,4-D at full bloom and petal drop (Mupambi, 2010) as well as at physiological fruit drop increased rind coarseness (Table 5.3). In addition to rind coarseness, a slight reduction in the juice content (%) of 2,4-D treated fruit was recorded, but no effect on other internal quality aspects were observed. This effect on internal fruit quality concurs with Mupambi (2010) and Stewart and Klotz (1947), who also reported a reduction in the juice content (%) of citrus fruit treated with 2,4-D. This slight reduction in juice content (%) is probably only due to the way juice % is calculated as the treated fruit had thicker rinds and actual juice content of sap vesicles was probably not affected.

To conclude, fruit splitting initiated at the stylar-end of 'Marisol Clementine' mandarin, the thinnest area of the fruit rind in January, and became visible in February. Application of 10 $mg \cdot L^{-1}$ 2,4-D after the physiological fruit drop period, both on its own and in combination with 5% Bonus-NPK, reduced fruit splitting. Both treatments increased (1) the thickness of the rind at the stylar-end of the fruit (2) lead to the formation of a solid, compact tissue at the stylar-end region of the fruit, (3) increasing the strength of the fruit rind. Both treatments changed (4) fruit shape (reduced d/l-ratio), making fruit less prone to split. However these 2,4-D treatments increased rind coarseness (enlarged oil glands), resulting in a reduction in appearance as well as slight reduction in juice percentage of

fruit at time of commercial harvest. Future studies should focus on elucidating the timing, rate, as well as cultivar sensitivity to 2,4-D applications to negate these negative aspects.

5.5. References

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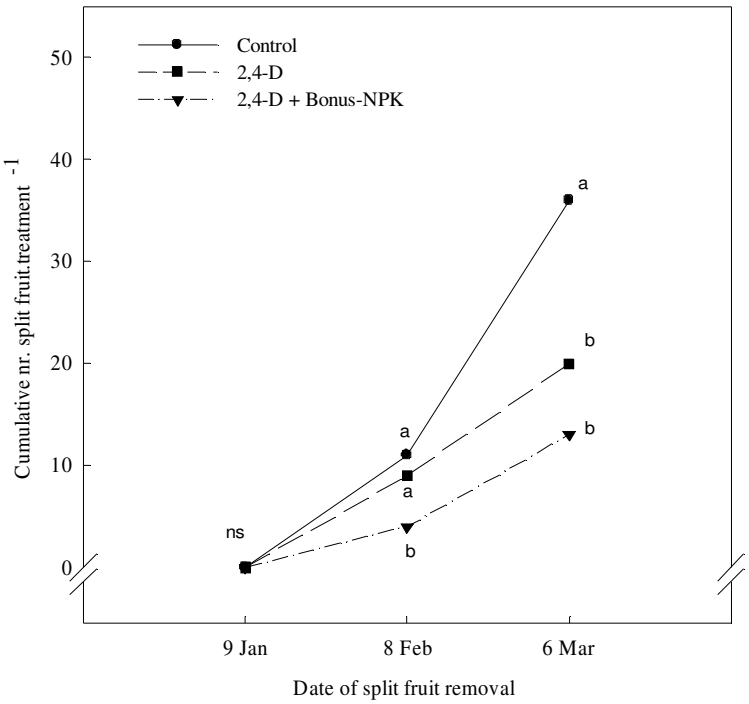

Fig. 5.1. The cumulative average number of split fruit per tree on 'Marisol Clementine' mandarin on 9 Jan., 8 Feb. and 6 Mar. 2012 in Paarl, South Africa. Treatments of 2,4-D (10 mg.L^{-1}) and 5% Bonus-NPK were applied directly after physiological fruit drop (average fruitlet diameter of 13 ± SE mm). Means with a different letter within an assessment date differ significantly at the 5% level.

^{ns} No significant differences

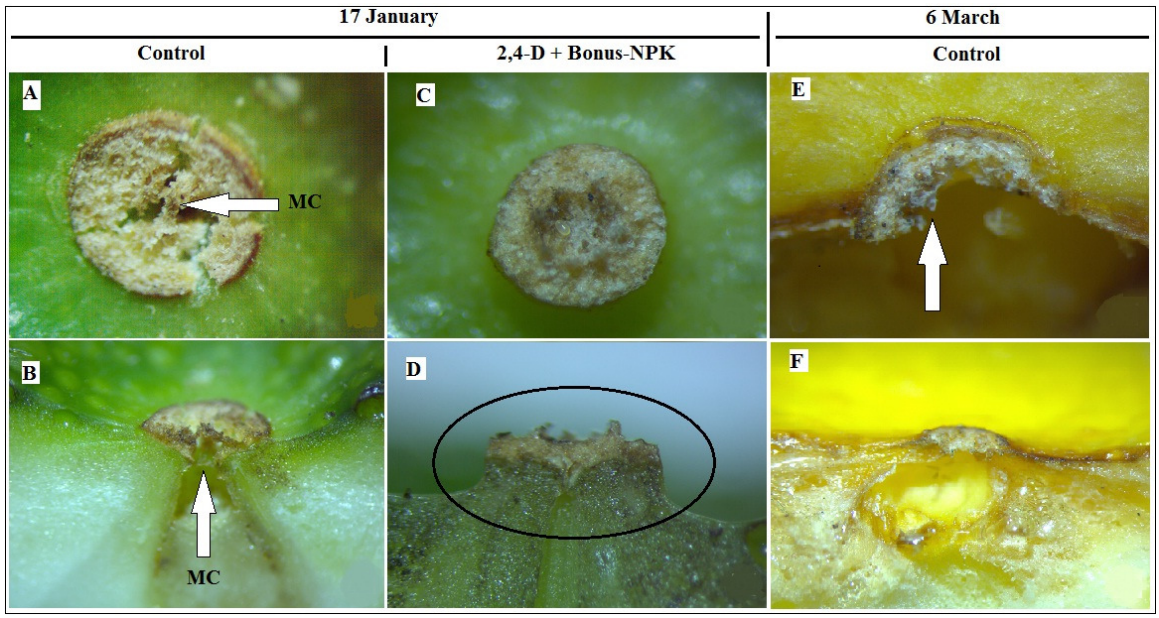

Fig. 5.2. Control fruit showing micro-cracks at stylar-end of fruit (MC), two weeks prior to visual fruit split initiation, on 17 January (A-B). No micro-cracks were visible at the stylar-ends of fruit treated with 2,4-D + Bonus-NPK on the same evaluation date in January (C-D). The apical floral meristem extended into the style of the fruit (D) (circle), which after style abscission, resulted in a compact, solid tissue. In March the split lesion (arrow) extended into the flavedo and albedo of the control fruit as the split became more severe (E-F).

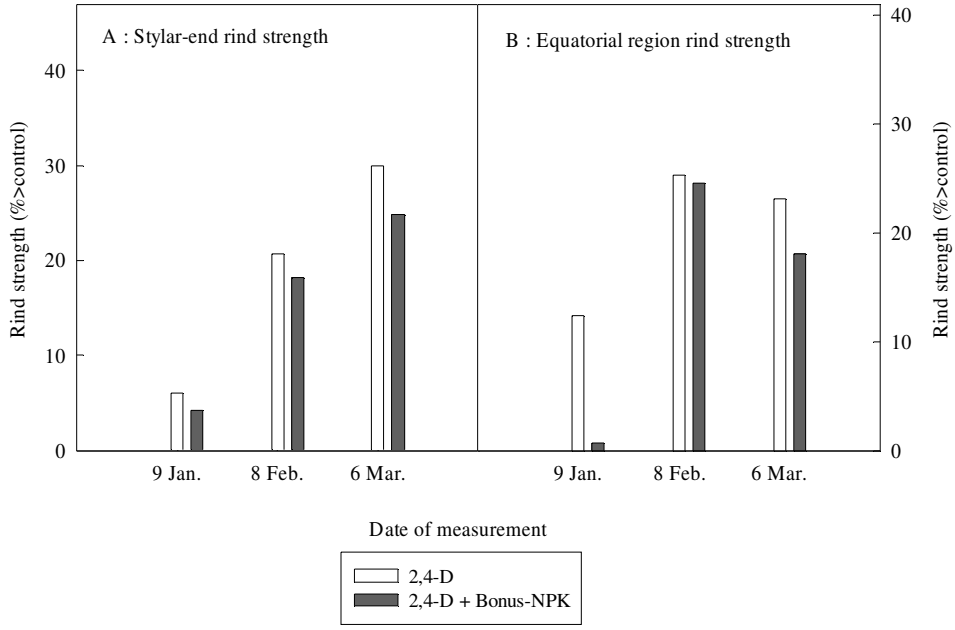

Fig. 5.3. Effect of 2,4-D and 2,4-D in combination with 5 % Bonus-NPK on (A) rind strength at the stylar-end and (B) at the equatorial region of treated fruit expressed as the increase in % compared to the control.

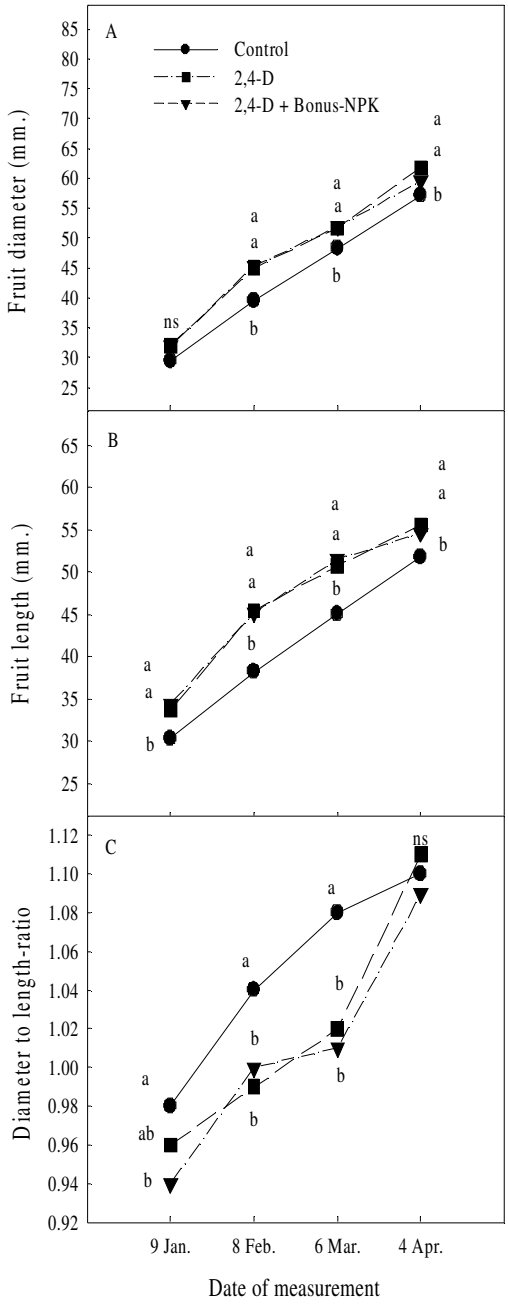

Fig. 5.4. (A) Average fruit diameter (mm) (B) fruit length (mm) and (C) diameter to length-ratio of 'Marisol Clementine' fruit treated with 10 mg·L^{-1} 2,4-D and 10 mg·L^{-1} 2,4-D + 5% Bonus-NPK after physiological fruit drop compared to untreated control fruit. Means with a different letter within an observation date differ significantly at the 5% level.
^{ns} No significant differences

Table 5.1: The influence of 2,4-D (10 mg·L^{-1}) and 5% Bonus-NPK foliar applications on rind strength at two regions of the fruit expressed as average peak force (N) required to cut the rind of 'Marisol Clementine', sampled at three different dates (2012) during fruit development.

Treatment	Rind Strength (N)					
	9 Jan.		8 Feb.		6 Mar.	
	Stylar-end	Equatorial region	Stylar-end	Equatorial region	Stylar-end	Equatorial region
Control	38.90b[z]	54.62[ns]	44.71b	42.68b	35.75b	39.78b
2,4-D*	41.42a	62.31	56.37a	57.31a	51.05a	51.17a
2,4-D + Bonus-NPK*	40.62ab	54.96	54.65a	56.62a	47.55a	48.54a
P-value	*0.0489*	*0.2610*	*0.0001*	*0.0034*	*0.0001*	*0.0010*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
* after physiological fruit drop
[ns] No significant differences

Table 5.2: The influence of 2,4-D (10 mg·L^{-1}) and 5% Bonus-NPK foliar applications as applied on various dates in 2012, on rind thickness (mm.) (means adjusted with diameter as covariate) of 'Marisol Clementine', at stylar-end as well as the equatorial region the fruit.

Treatment	Rind thickness (mm.)							
	Stylar-end				Equatorial region			
	9 Jan.	**8 Feb.**	**6 Mar.**	**4 Apr.**	**9 Jan.**	**8 Feb.**	**6 Mar.**	**4 Apr.**
Control	1.29 b[z]	1.96[ns]	1.31b	1.50[ns]	3.21[ns]	2.79[ns]	2.46b[z]	2.15[ns]
2,4-D*	1.36 b	2.44	2.04a	1.83	3.57	3.26	2.91a	2.47
2,4-D + Bonus-NPK*	1.84 a	2.59	2.07a	1.70	3.69	3.36	2.99a	2.34
P-value								
Treatment	*0.0039*	*0.0128*	*0.0004*	*0.0358*	*0.0039*	*0.0128*	*0.0004*	*0.0358*
Cov. (Diameter)	*0.2956*	*0.0072*	*0.0001*	*0.0021*	*0.2956*	*0.0072*	*0.0001*	*0.0021*
Treatment (adjusted)	*0.0137*	*0.4240*	*0.0104*	*0.0892*	*0.0637*	*0.4240*	*0.0104*	*0.0892*

[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
[ns] No significant differences
*after physiological fruit drop

Table 5.3: The influence of 2,4-D (10 mg·L^{-1}) and 5% Bonus-NPK foliar applications on rind coarseness of 'Marisol Clementine'.

Treatment	Rind coarseness (Score 1 to 4) [y]			
	9 Jan.	**8 Feb.**	**6 Mar.**	**4 Apr.**
Control	2[ns]	2 b[z]	2 b	2b
2,4-D*	2	3 a	3 a	3a
2,4-D + Bonus-NPK *	2	3 a	3 a	3a
P-value	*0.4648*	*0.0016*	*0.0193*	*0.0025*

[y] (1: smooth rind, 4: very coarse rind)
[ns] No significant differences
[z] Means with a different letter within a column differ significantly at the 5% level (LSD)
*after physiological fruit drop

Table 5.4: The influence of 2,4-D (10 mg·L^{-1}) and 5% Bonus-NPK foliar applications on internal fruit quality (means adjusted with diameter as covariate) of 'Marisol Clementine' evaluated at commercial harvest (2 April).

Treatment	Internal fruit quality			
	TA[x]	**°Brix**	**°Brix:TA**	**Juice content (%)**
Control	1.46[ns]	10.93 [ns]	7.68[ns]	49.12[ns]
2,4-D*	1.26	10.71	8.61	48.58
2,4-D + Bonus-NPK*	1.40	10.71	7.82	48.51
P-value				
Treatment	*0.0891*	*0.3160*	*0.0915*	*0.0012*
Cov. (Diameter)				*0.0091*
Treatment (adjusted)				*0.0602*

[x] Titratable acidity
[ns] No significant differences
* after physiological fruit drop

6. OVERALL DISCUSSION AND CONCLUSION

Citrus fruit splitting entails the initiation of cracks at the stylar- or navel-end of the fruit, developing into splitting and eventual abscission of affected fruit. Foliar applications of 2,4-dichlorophenoxy acetic acid (2,4-D) in combination with gibberellic acid (GA_3) and mineral nutrients i.e. calcium (Ca) and potassium (K), were evaluated for efficacy to reduce the disorder. The experiments were conducted over two consecutive seasons on five different split-prone cultivars, viz., Marisol Clementine, Mor and Orri mandarin, Midknight Valencia and Bahianina Navel. The application of 10 $mg \cdot L^{-1}$ 2,4-D directly after physiological fruit drop, either alone or in combination with K reduced splitting of 'Marisol Clementine' and 'Mor' mandarin by up to 50% in both seasons. Differences in sensitivity of cultivars to 2,4-D were evident, with the January application of 10 $mg \cdot L^{-1}$ 2,4-D reducing splitting in 'Midknight Valencia' orange and 'Orri' mandarin more effectively. No splitting occurred in 'Bahianina Navel' and treatment effect on fruit splitting in this orange cultivar could therefore not be evaluated.

Successful 2,4-D and K treatments in mandarin increased the thickness of the rind at the stylar-end of the fruit that lead to the formation of solid, compact tissue at the stylar-end region of the fruit, increasing the strength of the fruit rind. Both fruit diameter and –length increased to such an extent that the fruit shape was altered (reduced d/l-ratio). Rind coarseness of treated fruit was increased, but in most cases not significantly. No styles remained attached to the fruit, as reported in previous studies and the application of 2,4-D, after physiological fruit drop, allowed for successful natural style abscission to occur.

Growth rate ($mm.day^{-1}$) of all the mandarin cultivars was increased by a foliar application of 10 $mg \cdot L^{-1}$ directly after physiological fruit drop. The successful increase in fruit growth rate by 2,4-D application after physiological fruit drop is thought to be a result of a combination of an increase in rind thickness, as well as increased expansion of juice vesicles of the pulp, due to a possible increase in the sink strength for water and assimilates of treated fruit. However, the growth rate of orange cultivars was reduced by all the 2,4-D treatments.

Except for a slight reduction in juice content (%) and total acidity (TA), there were no effects on the °Brix, and °Brix:TA of all the cultivars at commercial harvest. This slight reduction in juice content (%) was probably only due to the way juice % was calculated as the treated fruit generally had thicker rinds and actual juice content of sap vesicles was probably not affected.

A medium cover foliar spray of 10 $mg \cdot L^{-1}$ 2,4-D directly after physiological fruit drop could be used for 'Marisol Clementine' mandarin and 'Mor' mandarin orchards with a history of severe fruit splitting. Similar application of 2,4-D in January on 'Midknight Valencia' orange is not recommended, due to the negative effect on fruit size and juice content.

This study offers proof that the post physiological fruit drop application of 2,4-D, between stage I and II of fruit development, increases rind thickness and -strength of mandarin and as a result reduces fruit susceptibility to the physiological disorder of the

rind that is fruit splitting. Future studies should, however also include a study of the effect of these 2,4-D treatment on yield/tree, as it was not evaluated in this study.

It is hypothesized that this particular action of 2,4-D application in December may be a remedy for other major citrus rind disorders of economic importance, such as creasing, which is caused by the excessive loss of cohesion between albedo cells and stressed by the expansion of the pulp during stage II of fruit development. Most of the rind disorders in citrus are a result of excessively thin and weak rind and the effect of December application of 2,4-D on these two important rind attributes could therefore aid in the control of rind disorders in citrus. However, it should also be stressed that, although mostly not significantly, 2,4-D applications used in this study lead to development of coarse rind, particular in mandarin cultivars, as a result of enlarged oil glands in the flavedo. It is therefore recommended that the effect of 2,4-D application directly after physiological fruit drop, should be evaluated for the incidence of oleocellosis, a skin injury caused by oil released from the oil glands in the skin after they have ruptured during harvest.