Fruit splitting of citrus (Citrus sp.) is a physiological disorder of the rind (Cronje et al., 2014) and occurs in thin-rinded mandarin and mandarin hybrids (Almela et al., 1994), as well as sweet orange (Citrus sinensis) cultivars such as Navel (De Cicco et al., 1988) and Valencia (Borrero et al., 1981). Similarities in certain causal factors and physiological development of splitting exist between citrus and other commercial fruit species; however, the unique anatomy and physiology of citrus fruit add some complexity to understanding and controlling this disorder.

Several factors impede citrus fruit development and result in the rind being more susceptible to split; that is, Ca and K deficiency (Bar-Akiva, 1975; De Cicco et al., 1988; Erickson, 1997), warm and humid climatic conditions during early fruit development (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).

Control strategies have focused on the use of plant growth regulators and nutrients as foliar treatments. Foliar application of 2,4-D has been shown to reduce fruit splitting in ‘Neva’ mandarin and various sweet orange cultivars (Almela et al., 1994; Borrero et al., 1981; Garcia-Luis et al., 2001) and to have a positive effect on fruit size (Agustí et al., 2002). However, in many instances, treatment results in development of enlarged oil glands in the fleshy, generally referred to as increased rind coarseness (Coggins and Hield, 1968). The reduction in fruit splitting in ‘Marisol’ following 15 and 25 mg L\(^{-1}\) 2,4-D applications during full bloom was accompanied by not only an increased rind coarseness but also attached styles at harvest, reducing the cosmetic appearance of treated fruit (Mupambi, 2010).

Foliar application of 2,4-D is thought to reduce splitting by increasing rind strength as seen by the increased resistance to puncturing in ‘Neva’ mandarin (Almela et al., 1994). In addition, 2,4-dichlorophenoxyacetic acid altered the fruit dimensions (increased fruit length), relieving the tension at the splitting prone stylar end of the fruit. By combining 2,4-D with K, the efficacy of 2,4-D was increased, resulting in larger fruit in both ‘Shamouti’ and ‘Valencia’ sweet orange (Erner et al., 1993), and reduced fruit splitting of ‘Neva’ mandarin (Greenberg et al., 2006).

The aim of this study was to evaluate the effects of foliar applications of 2,4-D on two split-prone mandarin cultivars at different phenological stages and at lower rates than previously reported, with the overarching aim of maintaining commercially acceptable internal and external fruit quality. The effect of 2,4-D foliar applications APFD on fruit growth rate was also evaluated. It is hypothesized that foliar application of 2,4-D APFD, either alone or in combination with Ca or K, may reduce the severity of citrus fruit splitting in ‘Marisol’ and ‘Mor’, without compromising fruit quality and tree health.

**Materials and methods**

**Spray material and application method.** The less volatile dimethyl amine salt formulation of 2,4-D [10 mg L\(^{-1}\) (Dow AgroSciences, Pretoria, South Africa)] was used to avoid any harmful drift of applications onto neighboring 2,4-D-sensitive crops.

2,4-dichlorophenoxyacetic acid was applied as a foliar spray on its own or in combination with K [Bonus N–P–K (Haifa Chemicals, Cape Town, South Africa), a fully soluble, crystalline formulation of potassium nitrate (K\(\text{NO}_3\)),...
with an analysis of 13N–0.8P–36.5K at 2.5% or 5%, or 2% Ca [a granular formulation of calcium nitrate (Ca(NO₃)₂)] (Omnia Nutriology, Bryanston, South Africa) with 155 g·kg⁻¹ nitrogen (N) and 195 g·kg⁻¹ Ca.

All foliar treatments were applied with a nonionic wetting agent (Break-Thru®, Villa Crop Protection, Kempton Park, South Africa) with the active ingredient polyether-polyalkylsiloxane-copolymer (1000 g·L⁻¹) at a rate of 5 mL per 100 L spray solution. Applications were made using a hand-gun sprayer until runoff, at ~10 L per tree and buffer trees were left untreated between trees in the orchard row. For all the control treatments, trees were sprayed directly APFD with water and the nonionic wetting agent.

**Plant Material and Treatments.** The experiment was conducted over two consecutive seasons (2010–11 and 2011–12) in commercial mandarin orchards (15–20 years old) of ‘Marisol’ and ‘Mor’ in Paarl, South Africa (lat. 33° 69′ S, long. 18° 95′ E), a citrus producing region in the Western Cape province that experiences Mediterranean climatic conditions. Trees in both orchards were budded onto ‘Carrizo’ citrange (C. sinensis × Poncirus trifoliata) rootstock. Foliar treatments for ‘Marisol’ were applied directly APFD ± 45 d after full bloom (DAFB) at an average fruitlet diameter of 13 to 17 mm, as well as at a later timing, at an average fruitlet diameter of 35 to 35 mm ± 90 DAFB (January) and 41 to 43 mm ± 120 DAFB (February). Foliar applications for ‘Mor’ were done directly APFD at an average fruitlet diameter of 15 ± 45 DAFB, as well as 23 to 25 mm ± 90 DAFB (January) and 34 to 36 mm ± 120 DAFB (February). Trees were chosen that were uniform in size and crop load, and the treatments were applied to the same trees during the following season.

**Data Collection.** Trees 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. Fruit splitting was expressed as the total number of split fruit removed and counted per tree and not as the percentage of the total fruit per tree, as the total yield per tree was not determined. In all the experiments, 10 fruit were randomly selected at a height of 1 to 3 m and tagged on trees receiving a 2,4-D application directly after the chemical treatments were applied. The diameters of these tagged fruit were measured at monthly intervals until harvest to calculate fruit growth rate (millimeters per day). During 2012, from ‘Marisol’ trees treated with 2,4-D and 2,4-D + K and the control, five fruit were picked per tree on 9 Jan. (before visual fruit split initiation), 8 Feb. (start of visual fruit split initiation), 6 Mar. (1 month after visual fruit split initiation), and 4 April (commercial harvest) for microscopic (ERc5s; Carl Zeiss, Göttingen, Germany) photographic comparisons of the stylar end, as well as rind strength determination. Rind strength was measured and expressed as rind cutting force (Newtons) by cutting two 10 × 10-mm pieces of the rind from opposite checks at the equatorial region and one from the stylar end of a five-fruit replicate. The rind pieces were then positioned on a texture analyzer (TAXT2i; Stable Microsystems, Surrey, 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 after calibrating the load cell to 250 N and cutting at a speed of 1 mm·s⁻¹. A 12-fruit sample was collected per tree at commercial harvest, six from each row-side to determine internal and external fruit quality. Rind coarseness was scored on a scale of one to four, with one being smooth and four being extremely coarse (enlarged oil glands of the flavedo). An electronic calliper (CD-6 C; Mitutoyo Corp, Tokyo, Japan) was used to measure fruit diameter and fruit length in millimeter. Fruit were cut into half along the longitudinal plane to measure rind thickness in millimeter with the electronic calliper at the equatorial region and stylar end, before being juiced to determine juice content (percentage), TSS (percentage) (PR-32 Palette electronic refractometer; Atago Co, Tokyo, Japan), and TA.

**Statistical Design and Analysis.** All treatments were applied in a randomized complete block design with 10 single-tree replicates per treatment. Buffer trees between treated trees as well as buffer rows were included in the trial layout. Statistical analyses of variance were performed using PROC GLM of the SAS (version 9.1; SAS Institute, Cary, NC). Mean separation was done by the least significant difference test, where applicable (P = 0.05).

**Results and Discussion.**

**Fruit Splitting.** In 2012, splitting was reduced in both cultivars by 2,4-D application compared with the control. Although a similar, but non-significant trend was evident in 2011, this reduction of fruit splitting in mandarin is in agreement with Greenberg et al. (2006) who reported lower incidence of splitting in ‘Nova’ mandarin following a 2,4-D application at 13 mm fruit diameter, albeit with a higher concentration of 40 mg·L⁻¹ 2,4-D, whereas Almela et al. (1994) reported successful reduction of splitting of ‘Nova’ mandarin with two tank-mix applications of 2,4-D (20 mg·L⁻¹) and GAs₃ (20 mg·L⁻¹) at later fruit developmental stages; that is, 30 and 60 d before initiation of fruit splitting.

For ‘Marisol’, fruit splitting was significantly reduced by application of 2,4-D at a similar stage in a tank-mix application with K (5%) in 2011 and 2012, as well as with a lower dosage of K (2.5%) in 2012 (Table 1), whereas only small differences between treatments occurred in 2011 for ‘Mor’. In 2012, APFD-application of 2,4-D alone, as well as the tank-mix with K (5%) and Ca, significantly reduced fruit splitting of ‘Mor’ compared with the control treatment (Table 1). In both cultivars, the positive impact of 2,4-D was reduced if applied in January, no significant effect obtained when delayed until February, and in some instances increased the number of split fruit (Table 1).

Foliar application of Ca was ineffective in reducing splitting, which is in contradiction with Almela et al. (1994) and Soodode and Chiarawipa (2005). However, in citriculture, the best strategy to improve Ca content of fruit is still to optimize root uptake and transport via the transpiration stream in the xylem as Ca is not readily absorbed from the fruit surface (Hanger, 1979).

**Fruit Development and Morphology.** For both cultivars, the application of 2,4-D APFD increased fruit growth rate significantly in comparison with the control (Table 2), whereas the later applications of 2,4-D had no or little effect, and in some instances reduced fruit growth rate.
Table 1. The effect of 2,4-dichlorophenoxyacetic acid (2,4-D), potassium (K), and calcium (Ca) applied alone or combined as foliar treatments [10 L (2.6 gal) per tree] at different timings [after physiological fruit drop (APFD), January, and February] on total fruit splitting of two mandarin cultivars (Marisol and Mor) in 2011 and 2012 at Paarl, South Africa (lat. 33°69’S, long. 18°95’E).

| Chemical | Conc of chemical | Timing of treatment | ‘Marisol’ 2011 | ‘Mor’ 2011 | ‘Marisol’ 2012 | ‘Mor’ 2012 |
|----------|-----------------|---------------------|---------------|------------|---------------|------------|
| Control  | —               | —                   | 209 abc        | 8 ab       | 68 a          | 31 a       |
| 2,4-D    | 10 mg L⁻¹       | APFD                | 121 cd         | 3 b        | 33 b          | 15 c       |
| K        | 2.5%            | APFD                | —             | 42 b       | —             | —          |
| 2,4-D + K| 10 mg L⁻¹ + 2.5%| APFD                | 154 bcd        | 5 ab       | 33 b          | 26 a       |
| 2,4-D + Ca| 10 mg L⁻¹ + 2% | APFD                | 237 ab         | 9 a        | —             | 23 ab      |
| 2,4-D    | 10 mg L⁻¹       | January             | 79 d           | 7 ab       | 18 b          | 13 c       |
| 2,4-D    | 10 mg L⁻¹       | February            | 151 bcd        | 5 b        | —             | 13 c       |
| 2,4-D    | 10 mg L⁻¹       | January             | 273 a          | 8 a        | —             | 20 b       |
| 2,4-D    | 10 mg L⁻¹       | February            | 184 bcd        | 7 ab       | —             | 26 a       |
| P value  |                 |                     | 0.0038         | 0.0140     | 0.0021        | 0.0001     |

*Nonionic wetting agent with 1000 g L⁻¹ (133.5 oz/gal) of polyether-poly(methyloxysiloxane) polymer (Break-Thru®, Villa Crop Protection, Kempton Park, South Africa) was applied with every chemical at 5 mL per 100 L (0.64 fl oz/100 gal) spray solution.

1 mg L⁻¹ = 1 ppm.

*Means with a different letter within a column significantly at the 5% level (least significant difference).

In 2011, there were no significant differences in fruit diameter of ‘Marisol’ between the single 2,4-D, 2,4-D + K (5%) and the control at the first evaluation date on 9 Jan. (Fig. 1A). Thereafter, both treatments resulted in significantly larger fruit diameter in February, March, and April than the control treatment, which concurs with El-Otmani et al. (1993) and Greenberg et al. (2006). The increase in fruit size by 2,4-D could be due to a dual action whereby it increases the rind thickness, as well as juice vesicle cell expansion (El-Otmani et al., 1993). 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 APFD.

Fruit length was significantly longer for both foliar sprays in comparison with the control, on all the evaluation dates [January to April (Fig. 1B)]. The diameter:length ratio differed significantly between the two treatments and the control in February and March, with the control fruit having higher ratio values, indicating these fruits were more oblate than the 2,4-D-treated fruit. Considine and Brown (1981) showed that the tension on fruit pericarp tissue exerted at the polar regions (stylar- and stem-end) is highest, 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 K increased fruit length (Fig. 1), making fruit less oblate and thereby possibly relieving the tension exerted on the stylar end of the fruit, and reducing the susceptibility to fruit splitting.

Stimulation of cell expansion by 2,4-D treatments (Mitchell, 1961...
may have been responsible for the increase in rind strength of treated fruit, as the application of 10 mg·L⁻¹ 2,4-D on ‘Marisol’ resulted in a significantly stronger rind. Rind strength at the stylar end of the fruit was increased by 2,4-D treatments in comparison with the control, ranging from 5% to 7% in January, 18% to 20% in February, and 25% to 30% in March (Fig. 2), concurring with a similar effect obtained on ‘Nova’ mandarin by Almela et al. (1994).

The formation of a uniform abscission layer without tears between the style and stylar end of the rind seems to be important to prevent stylar-end fruit splitting, as the small lesion is thought to be the starting point for this disorder. Natural auxins in plant tissue such as 3-indoleacetic acid 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 APFD as reported here are thought to have allowed for a more gradual style abscission to occur. Visual evaluation of the stylar end region of 2,4-D-treated fruit revealed the rind to be slightly elevated, thickened, and without microcracks (Fig. 3). Stemming from these observations, it is thought that the treatment could 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. 3C and D). In control fruit, the floral axis did not extend toward the style and after abscission of the style, a cavity was formed instead of solid rind tissue (Fig. 3A and B), which could possibly compromise rind integrity.

**Fruit Quality.** The thinner stylar-end fruit rind in 2011 (1.30 mm) compared with 2012 (1.50 mm) (Table 3) could partly explain the higher severity of the disorder in 2011. 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. Environmental and cultural practices have an effect on rind thickness and are related to split incidence; that is, very warm and humid conditions (Almela et al., 1994), as well as mineral nutrients and water stress (De Cicco et al., 1988). In 2011, for ‘Marisol’, rind thickness at the stylar end was not significantly affected by any of the treatments except K (5%), while rind thickness at the equatorial region was decreased by Ca (Table 3). However, in 2012, all the treatments increased fruit rind thickness at the stylar end, except K (5%), and also at the equatorial region of the fruit with the exception of the tank-mix application of 2,4-D and K (2.5%) (Table 3). For ‘Mor’, all treatments with Ca or K, as well as when tank-mixed with 2,4-D significantly increased rind thickness at the stylar end, whereas 2,4-D applied alone directly APFD increased rind thickness at the equatorial region of the fruit in 2012 (Table 3).

In citriculture, preharvest treatment with 2,4-D at very high concentrations (>20 mg·L⁻¹) lead to the
Fig. 2. The effect of a foliar application of 10 mg L\(^{-1}\) (ppm) 2,4-dichlorophenoxyacetic acid (2,4-D) and 10 mg L\(^{-1}\) 2,4-D + 5% potassium (K applied as 13N–0.8P–36.5K) after physiological fruit drop, on (A) rind strength at the stylar end of ‘Marisol’ clementine mandarin and (B) at the equatorial region of treated fruit in 2012, expressed as the percentage increase over control, untreated fruit. Rind strength was measured by cutting two 10 x 10-mm pieces of the rind from opposite cheeks at the equatorial region and one from the stylar end of a five-fruit replicate. The rind pieces were then positioned on a texture analyzer (TAXT2i; Stable Microsystems, Surrey, 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 after calibrating the load cell to 250 N (56.2 lbf) and cutting at a speed of 1 mm s\(^{-1}\); 1 mm = 0.0394 inch.

Fig. 3. Control fruit showing microcracks (MC) at the stylar end of ‘Marisol’ clementine mandarin fruit, 2 weeks before visual fruit split initiation, on 17 Jan. 2012 (A and B). No microcracks were visible at the stylar ends of fruit treated with a foliar application of 10 mg L\(^{-1}\) (ppm) 2,4-dichlorophenoxyacetic acid (2,4-D) + 5% potassium (K applied as 13N–0.8P–36.5K) after physiological fruit drop, on the same evaluation date. The apical floral meristem extended into the style of the fruit (C and D) (circle), which after style abscission, resulted in a compact, solid tissue, making the treated fruit less prone to fruit splitting. On 6 Mar. 2012, the split lesion on the control fruit (arrow) extended into the flavedo and albedo, which eventually manifested as fruit splitting (E and F).
development of excessively thick and coarse rinds of treated fruit due to enlarged oil glands in the flavedo (Stewart et al., 1951). The synthetic auxin 2,4-D is rapidly absorbed and translocated in the phloem to young meristematic tissue and accumulates in sink organs such as young leaves, flowers, or fruitlets where it stimulates cell expansion (Ashton and Monaco, 1991). Mupambi (2010) found that applications of 25 mg L⁻¹ at full bloom, as well as 15 and 25 mg L⁻¹ at petal drop reduced split of ‘Marisol’, but increased rind coarseness. However, with the lower concentration (10 mg L⁻¹) and later timing (APFD) of 2,4-D application reported in this study, none of the treatments had a significant effect on rind coarseness in any season (data not shown).

There were no significant differences in the TSS, TA, and TSS:TA ratio between treated or control fruit, and although not significant, it was noted that all the treatments with 2,4-D marginally reduced juice content of the fruit (data not shown).

**Conclusion**

Foliar application of 10 mg L⁻¹ 2,4-D directly APFD reduced the number of split fruit in ‘Marisol’ by 42% in 2011 and 50% in 2012. In ‘Mor’, the number of split fruit was reduced by 63% in 2011 and 50% in 2012. In addition, foliar application APFD of 2,4-D and 2,4-D + K treatments increased rind thickness at the stylar end of the fruit resulting in a solid, compact tissue at the stylar-end region of the fruit and therefore increasing the strength of the fruit rind. In addition, successful treatments modified the fruit shape (reduced diameter:length ratio), resulting in less split-prone fruit and increased the growth rate (millimeters per day, measured from fruit diameter) of ‘Marisol’ and ‘Mor’ significantly. Successful increase in growth rate by 2,4-D application APFD 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. Except for a slight, but nonsignificant reduction of juice content and TA, there was no commercially significant negative effect of the treatments on TSS and TSS:TA ratio. A single, medium-cover application of 10 mg L⁻¹ 2,4-D timed directly APFD can be used in ‘Marisol’ and ‘Mor’ orchards with a history of severe fruit splitting, especially in years with heavy fruit load.

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