

# Effect of 2,4-D exogenous application on the abscission and fruit growth in Sweet orange. var. Salustiana

## Efecto de la aplicación exógena de 2,4-D en la abscisión y crecimiento del fruto de naranjo dulce var. Salustiana

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### ABSTRACT

The effect of 2,4-D applications in full bloom on the abscission and fruit growth process was studied on sweet orange fruit in 20-year-old trees of *Citrus sinensis* (L.) Osbeck cv. Salustiana with a high flowering level. Abscission was determined on the whole tree and on the leafy inflorescences. Growth variables of the fruit were characterized (diameter, fresh and dry weight). 2,4-D application (20 mg L<sup>-1</sup>, 3.6 L per tree) increased the growth rate of the fruits and fruits size at maturity, however reduced the number of fruits which kept constant the yield at harvest. Differences between the diameter of the control fruits and the fruits treated with 2,4-D were observed during the early fruitlet development and until day 43 after anthesis. These differences increased with time following a linear relationship. For all the studied variables the diary increase level reaches the maximum by day 53, when the cell expansion of the vesicles starts.

**Key words:** *Citrus sinensis*, flowering, set fruit, fruit growth and development.

### RESUMEN

Se evaluó el efecto de la aplicación de 2,4-D en floración completa sobre los procesos de abscisión y crecimiento del fruto del naranjo dulce *Citrus sinensis* (L.) Osbeck de la variedad Salustiana, en árboles de 20 años de edad con un alto nivel de floración. Se determinó la abscisión en todo el árbol, al igual que en inflorescencias con hojas, caracterizando las variables de crecimiento del fruto (diámetro, peso fresco y peso seco). La aplicación de 2,4-D (20 mg L<sup>-1</sup>, 3.6 L por árbol) incremento la velocidad de crecimiento del fruto y su tamaño final al momento de la cosecha, reduciendo su número pero sin modificarla. Se presentaron diferencias en diámetro en relación con el testigo sin tratar, durante el desarrollo temprano del fruto y hasta el día 43 después de anthesis. A partir de este momento estas diferencias se incrementaron con el tiempo. La tasa de aumento diaria para todas las variables estudiadas alcanzó un máximo el día 53, momento en que dio inicio el crecimiento en expansión de las vesículas.

**Palabras clave:** *Citrus sinensis*, floración, cuajado del fruto, crecimiento y desarrollo del fruto.

## Introduction

The sweet orange *Citrus sinensis* (L.) Osbeck variety Salustiana is a parthenocarpic cultivar (González-Sicilia, 1968). Parthenocarpic cultivars usually show lower fruit set and fruit size than seeded cultivars. However, many citrus cultivars produce a high number of flowers that are enough to obtain a high yield. Flower number is inversely correlated to the percentage of the final fruit set and fruit size (Goldschmidt and Monselise, 1977; Agustí *et al.*, 1981; Becerra and Guardiola, 1984).

Citrus trees show two successive abscission waves that affect flowering and fruit development. In some cases the abscission is a continuous process with the highest peak between 6 and 8 weeks after full flowering (Duarte and Guardiola, 1996; Laskowski, 2006). The first wave induces

a massive abscission of flowers and ovaries while the second reduces the fruit number, reaching a significant growth during June drop (Goldschmidt, 1999; Guardiola, 2000; Goren *et al.*, 2000; Iglesias *et al.*, 2006; Pérez and Jiménez, 2009).

The abscission can appear in the flowers and ovaries around the pedicel zone (AZ-A) while in the developing fruit during June drop can appear around the calyx (AZ-C) (Schneider, 1968; Spiegel-Roy and Goldschmidt, 1996; Guardiola and García-Luis, 2000; Iglesias *et al.*, 2006; Laskowski *et al.*, 2008; Pérez and Jiménez, 2009). In both cases the abscission is mediated by ethylene synthesis (Ortolá *et al.*, 1991; Burns *et al.*, 1992; Goren, 1993; Iglesias *et al.*, 2006). Some authors had suggested in oranges an increase in fruit abscission by a direct or indirect stimulation in the ethylene production (Goren, 1993; Kazokas and Burns, 1998).

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The first abscission wave does not seem to be correlated with the supply of carbohydrates. The reserves of carbohydrates in the tree stay high during the first abscission wave (García-Luis *et al.*, 1988; Erner, 1989; Ruiz and Guardiola, 1994; Ruiz *et al.*, 2001). Fruit drop in the early stages of fruit development in the first abscission wave is preceded by a reduction in the growth rate. The reduction in the transport of carbohydrates is related with lower sink strength and not with the supply of metabolites (limitation at the source) (Ruiz and Guardiola, 1994; García-Luis *et al.*, 2002).

In citrus, the sink strength of the developing fruit as well as yield of commercially valuable large-size fruit increases with exogenous auxin applications (Guardiola and García-Luis, 2000; Chao and Lovatt, 2010). The result is a faster fruit growth until ripening (Guardiola and Lázaro, 1987; Ortolá *et al.*, 1988; Agustí *et al.*, 1994). The *in vivo* responses to exogenous auxin applications have been correlated with *in vitro* behaviour of fruit tissue. Guardiola *et al.* (1993) provided evidence that the sensitivity of the fruit tissues to growth regulators changes markedly during early fruit development. Studies made by Laskowski *et al.* (2008) demonstrated a strong correlation of higher endogenous indole acetic acid content with the growth rate in the Salustiana variety during early fruit development.

The greatest response had been obtained when auxin was applied during the flowering period or short after (Coggin and Hield, 1968; Duarte and Guardiola, 1996). In Clementine Esbal mandarin, application of 2.4-D during flowering increased the fruit growing rate and delayed the abscission more intensely several weeks after application (Duarte and Guardiola, 1996).

Reduction in fruit number was compensated with an increased in fruit size. A similar response was found in Nova mandarin (Guardiola, 1996). In several orange cultivars 2.4-D application was effective in increasing fruit size. This response was obtained when the application was made 6-8 weeks after flowering (Erner *et al.*, 1993). Applications of 2.4-D during the flowering period were not effective. We have found evidence that applications of 2.4-D during flowering increased fruit size in sweet oranges of the Salustiana cultivar without affecting the yield. The object of this study was to evaluate the effect of 2.4-D on the abscission and fruit development in Sweet orange. var. Salustiana.

## Materials and methods

### Plant material

The study was carried out in Valencia (Spain) and conducted on adult trees (20 years old) of Salustiana sweet orange

(*Citrus sinensis* (L.) Osbeck) grafted on rootstock citrange Troyer (*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.). The trees displayed alternate bearing and its flowering intensity was determined by the previous flowering. In the orchard in a same year, trees with different flowering intensities were often present.

### Hormone treatment

The application of 2.4-D was made once the trees showed 50% of flowers in anthesis on single and multi flowered leafy inflorescences. The 2.4-D application (Viriman, 10% (p/v) solution of isopropyl ester) was made on 11 trees. The concentration was 20 mg L<sup>-1</sup> and 3.6 L/tree. The application was made to the entire tree foliar surface. A non-ionic wetting agent, alkyl polyglycol ether, at a final concentration of 20% (p/v) was added to all solutions

### Abscission and fruit growth

Abscission and fruit growth studies were conducted on single and multiple-flowered leafy inflorescences. Hundred inflorescences of each type were tagged. The multiple-flowered leafy inflorescences had five leaves and flowers respectively. It was taken into consideration that the final flower bud of each inflorescence was next to open. Anthesis took place 7 d later. Fruit number of each inflorescence was recorded every 10 d. These records were used to calculate the relative and absolute abscission rate. Before the flower buds appeared, a canvas was installed underneath each tree. The flowers and developing fruit that fell on the canvas were collected periodically to determine the tree total abscission.

The fruit variables were fresh and dry weight and diameter. Measurements were made every 10 d when data were recorded. Fruit diameter was determined measuring the equatorial region of the apical fruit in each inflorescence. Twenty fruits were collected in order to determine fresh and dry weight and taking into reference the fruit diameter of the tagged inflorescences.

### Statistical analyses

The characteristics of the inflorescences, as related to fruit abortion, were compared using an ANOVA. The statistical package IBM®-SPSS (IBM Corporation, New York, NY) was utilised throughout. Statistical analyses include DMS and DUNCAN.

## Results

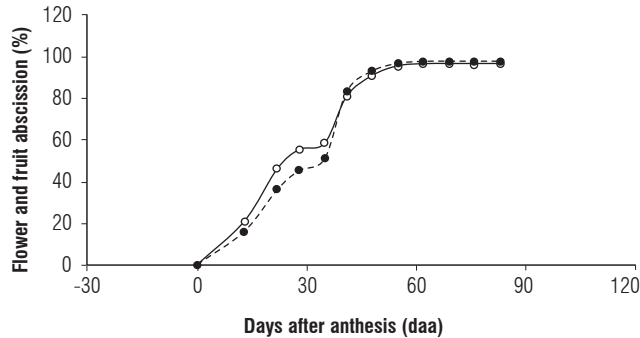
### Effect of 2.4-D on abscission pattern

The 2.4-D applications produced a reduction in the fruit number with an increase in the average weight without

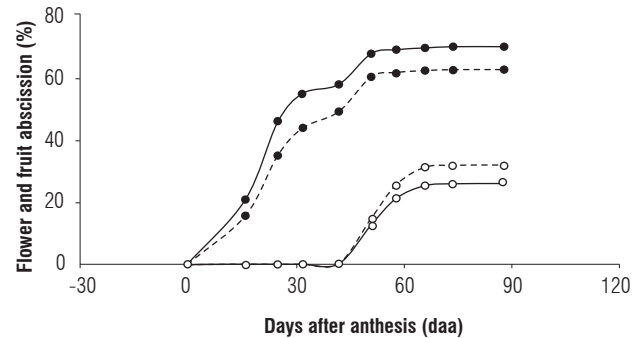
**TABLE 1.** Behaviour of the yield on 2.4-D treated and untreated 'Salustiana' trees.

Fruit weight (g)		Yield (kg)		Fruit number/tree	
Control	2.4-D	Control	2.4-D	Control	2.4-D
115±7 b	133±5 a	180±11 a	192±14 a	2,088±232 a	1,551±159 b

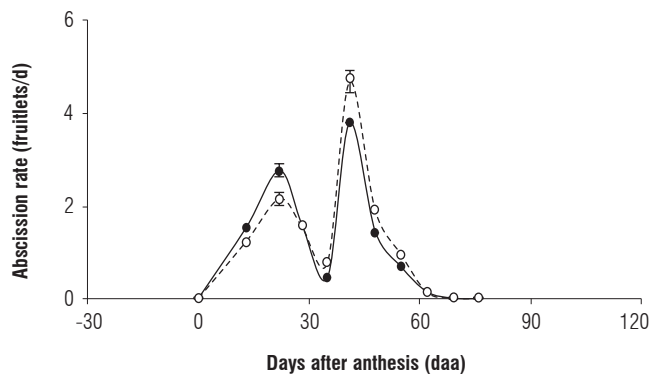
Mean values followed by distinct letters are significantly different according to Duncan's test ( $P \leq 0.05$ ).



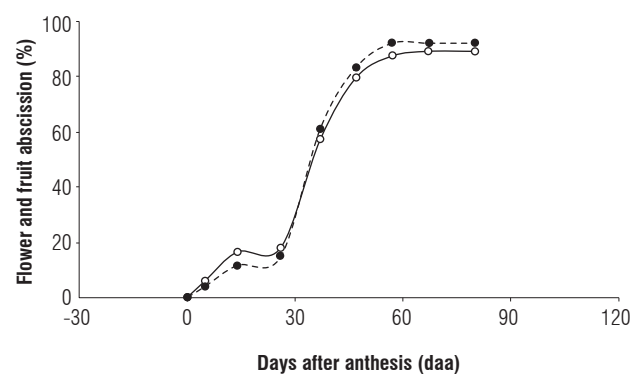
**FIGURE 1.** Accumulated abscission in 2.4-D treated (○) and untreated (●) 'Salustiana' trees.



**FIGURE 2.** Accumulated abscission of flowers and fruitlets in 2.4-D treated (---) and untreated (—) 'Salustiana' trees according to the abscission zone. Pedicel abscission zone (●) and calyx abscission zone (○).



**FIGURE 3.** Daily absolute (fruitlets/d) abscission rates in 2.4-D treated (○) and untreated (●) 'Salustiana' trees.



**FIGURE 4.** Accumulated abscission in multiple-flowered leafy inflorescences on 2.4-D treated (●) and untreated (○) 'Salustiana' trees.

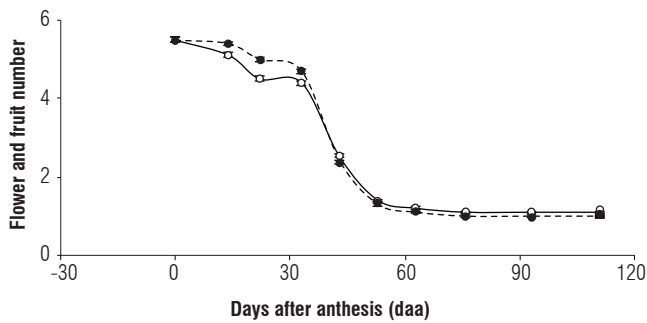
affecting the yield ( $P \leq 0.03$ ; Tab. 1). Also the 2.4-D applications produced a delay in the abscission. Until 42 daa (days after anthesis), the abscission was lower in the treated trees. From 42 daa, an increase in the accumulated abscission with a final fruit set of 3.1% in comparison with 3.7% in the control trees was observed (Fig. 1).

Abscission occurred in two periods. The first period is related to flowers and fruit that fall only at the pedicel. This was presented from anthesis to 42 daa. The second period occurred between 42-74 daa. This period is related to fruit that fall from both the pedicel and calyx base (Fig. 2). The change in the abscission pattern with 2.4-D applications was reflected as a differential effect in the abscission of both the pedicel and the calyx base. Until 42 daa the abscission by the pedicel was higher in control trees. From 42 to 67 daa the abscission was presented by the calyx base being

higher in the treated trees. Between 42 and 52 daa a transition period was presented with fruits that fall by both abscission zones.

In absolute terms (number of flowers/fruitlets shed per day), two main peaks of abscission occurred. The first one at 22 daa with 2.8 and 2.1% in untreated and 2.4-D treated trees respectively. The second main peak occurred at 42 daa with 3.8 and 4.7% in untreated and 2.4-D treated trees respectively (Fig. 3).

In multiple flowered leafy inflorescences, the abscission was slow until 22 daa. After 22 daa a considerable increase in the abscission was observed (Fig. 4). Differences in the time in which abscission stopped taking place were observed with 63 daa and 53 daa in untreated and 2.4-D treated trees respectively (Fig. 4).



**FIGURE 5.** Reduction in the fruit number per inflorescence in multiple – flowered leafy inflorescence on 2.4-D treated (●) and untreated (○) ‘Salustiana’ trees.

The abscission pattern was correlated with the reduction in the fruit number per inflorescence. Until 33 daa a reduction from 5.5 fruit per inflorescence to 4.4 in untreated trees, and 4.7 in 2.4-D treated trees was observed. From this day the reduction increased to stabilized at 63 daa with an fruit average of 1.2 in untreated trees and 1 in 2.4-D treated trees (Fig. 5).

There was a different behaviour in the percentage of inflorescences that retain at least one fruit. The reduction in the number of multiple-flowered leafy inflorescences that retain at least one fruit occurred from 26 daa while in single flowered leafy inflorescences this occurred immediately after anthesis. In both inflorescence types this parameter stopped at 67 daa. The 55% of multiple-flowered leafy inflorescences retain at least one fruit in comparison with the 39% in single flowered leafy inflorescences (Fig. 6).

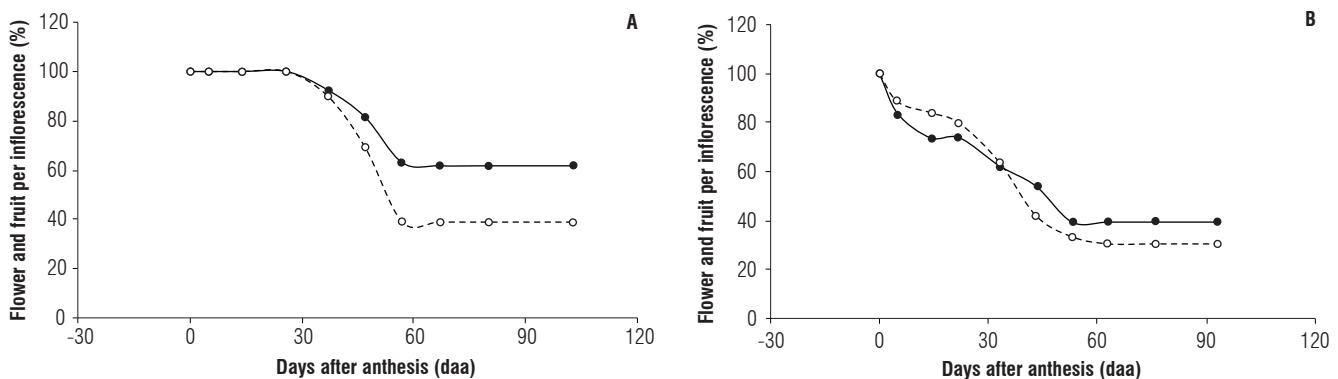
The 2.4-D applications increased the percentage of multiple-flowered leafy inflorescences that retain at least one fruit. Between 33 and 53 daa this parameter increased to reach the maximum with 55% in untreated and 36% 2.4-D treated trees (Fig. 6). In single flowered leafy inflorescences

a delay occurred until 33 daa, with a first peak in the relative and absolute abscission rate of 2% in untreated and 1.1% in 2.4-D treated trees. From this day the abscission increased in treated trees to stop at 63 daa in untreated trees with 61% while in 2.4-D treated trees it stopped at 76 daa with 70% (Fig. 6).

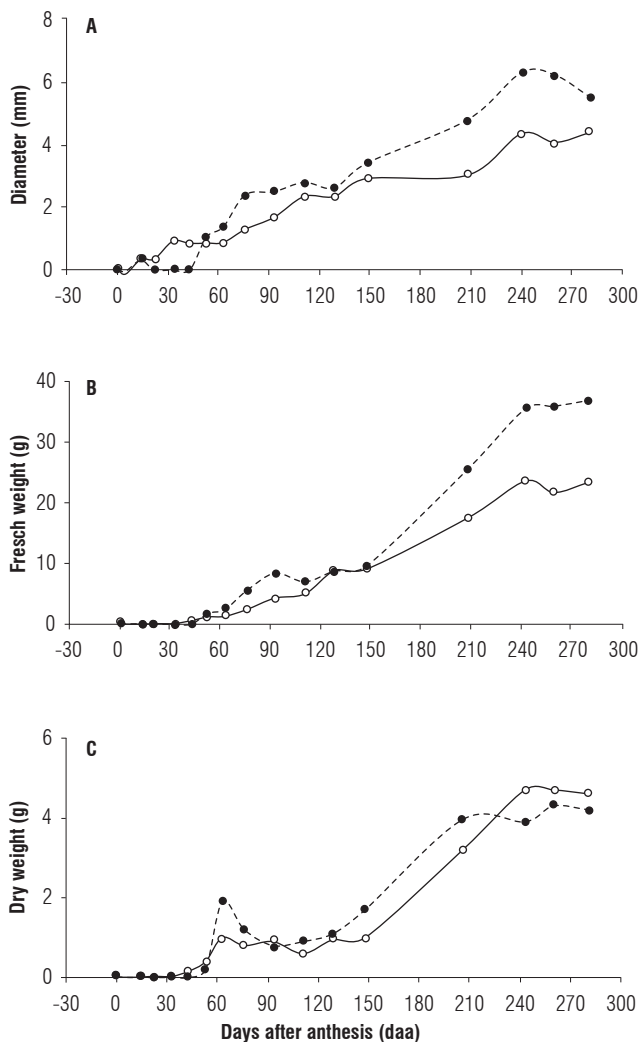
### Effect of 2.4-D on fruit growth pattern

The 2.4-D applications increased fruit size. The effect on the growth variables was observed immediately after anthesis. The differences between untreated and treated trees increased with the advance in the fruit developing stage. At time of harvest, the differences in diameter were of 5 mm in fruit of a single flowered leafy inflorescences and 4 mm in multiple-flowered leafy inflorescences (Fig. 7a). There was a higher effect on fruit from single flowered leafy inflorescences than multiple-flowered leafy inflorescences.

The increase in fresh weight was low throughout early fruit growth until 43 daa. From this day a lineal increase was observed until 240 daa as well as in the diameter. At the time of harvest, the differences in fresh weight were of 35.5 g in fruit of single flowered leafy inflorescences and 24 g in multiple-flowered leafy inflorescences, being higher in fruit treated with 2.4-D (Fig. 7b). Like the fresh fruit weight, the dry matter accumulation was low in the early fruit growth period until 43 daa. The differences during this time were kept close to zero. In fruit of single flowered leafy inflorescences there was a first period of slow accumulation of dry matter between 53 and 133 daa. In fruit of multiple-flowered leafy inflorescences this period was extended to 148 daa. From this day there was an increased, to stabilize at 198 daa in the first inflorescence type (single flowered leafy) and 40 d later in the other inflorescence type (Fig. 7c).



**FIGURE 6.** Percentage of inflorescences with one fruit of multiple (A) and single (B) flowered leafy inflorescence on 2.4-D treated (○) and untreated (●) ‘Salustiana’ trees.



**FIGURE 7.** Difference in diameter (A), fresh (B) and dry (C) weight between 2.4-D treated and untreated 'Salustiana' trees in single (●) and multiple (○) flowered leafy inflorescence.

## Discussion

The early drop of flower and ovaries after flowering time occurs by abscission of the pedicel zone (AZ-A). Once this zone becomes inactive, the calyx abscission zone (AZ-C) is activated during the June drop (Schneider, 1968; Spiegel-Roy and Goldschmidt, 1996; Guardiola and García-Luis, 2000; Iglesias *et al.*, 2006). In both cases the abscission is mediated by the ethylene synthesis (Goren, 1993; Ortolá *et al.*, 1997; Burns *et al.*, 1998; Iglesias *et al.*, 2006). The endogenous hormonal balance in the fruit controls the abscission process, especially auxins and ethylene. An additional contribution of auxins to the fruit can change the abscission process depending on the auxins concentration and type, as well as the fruit developing stage and type of

cultivar (Guardiola and García-Luis, 2000). Applications of 2.4-D produced a delay in the abscission until 42 daa reducing the drop of flower and small developing fruit of the AZ-A and increasing fruit drop of the AZ-C. This pattern was found also on Clementine Esbal mandarin (Duarte and Guardiola, 1996) as well as on 'Owari Satsuma' (Ortolá *et al.*, 1997). After the application of the auxins, the abscission delay occurs by the inhibition of enzymes that degrade the cell wall in the abscission zone (Goren, 1993; Goren *et al.*, 2000). Phenoxyacetic auxins were found to be more effective in delaying abscission (Ortolá *et al.*, 1997).

Although 2.4-D applications produced a reduction in the fruit set, the increase in fruit weight compensated this reduction without affecting the yield. This behaviour was previously reported in Clementine Esbal mandarin (Duarte and Guardiola, 1996) and Nova mandarin (Guardiola, 1996). In Valencia and Shamouti orange cultivars, 2.4-D applications were not effective in increasing the fruit size (Erner *et al.*, 1993). A higher response was obtained both in fruit set and fruit size when the applications were made 6-8 weeks after anthesis.

The increase in the fruit sink strength produced by the auxins applications is reflected in a faster growth of the fruit until ripening (Guardiola and Lázaro, 1987; Ortolá *et al.*, 1988; Agustí *et al.*, 1994). In several cultivars, the maximum response had been obtained when the auxin application was made during or after flowering (Coggins and Hield, 1968; Duarte and Guardiola, 1996). Most studies had focused in the characterization of the response of several mandarin cultivars to hormonal treatments before June drop (Guardiola and Lázaro, 1987; Ortolá *et al.*, 1991; Agustí *et al.*, 1991; Georgiu, 1998).

The effect on fruit growth when 2.4-D is applied at flowering time coincides with peak values in the endogenous content of IAA (Laskowski *et al.*, 2008). This effect was verified with the histological development pattern in different developing stages of the fruit. The maximum cell division rate in the explants was found in 6 d-old-fruits that produced callus with higher IAA concentration (Rebolledo *et al.*, 2007). The cell types of the callus were a key characteristic of the *in vitro* development pattern. In the untreated explants the callus cells were large, elongated and vacuole shaped. In the IAA treated explants the cell callus was small. This behaviour was related with the IAA action on cell division (Krikorian, 1995; Khan, 1996; Laskowski *et al.*, 2008; Rebolledo *et al.*, 2007).



There was a reduction in the capacity response when mesocarp explants were used. The mesocarp explants of 46 d-old-fruits showed a higher response with a minor concentration (10<sup>-6</sup> M) than explants of 61 d-old-fruits that only were affected for the higher concentration of IAA (10<sup>-5</sup> M). At 46 daa cell division activity was still observed in the external mesocarp (Amo-Marco and Picazo, 1994; Rebolledo *et al.*, 2007).

This activity can explain the effect of IAA on the callus growth. On 76 d-old-fruits the explants weren't affected by the evaluated IAA concentrations. With the advance in the developing stage of the mesocarp there were only differentiated, vacuole-shaped and polygonal cells with large intercellular spaces.

Applications of 2,4-D increased fruit size in the Sweet orange Salustiana cultivar. This effect was evident immediately after anthesis and until harvest time when there was a difference of 5 mm in fruit size. This behaviour was previously reported in Clementine Esbal mandarin with the same 2,4-D concentrations used in this study (17 and 20 mg L<sup>-1</sup>). The same effect was reported in Nova mandarin cultivar with a reduction in the final harvest (around 10%) (Guardiola, 1996).

Applications of 2,4-D increased the fruit growth rate. The diameter differences between fruit treated and untreated with 2,4-D on single flowered leafy inflorescences and multiple-flowered leafy inflorescences were established from fruit early development. These differences increased linearly with the advance in the developing stage. During the fruit early development (43 daa) the fresh weight increased and the dry matter accumulation showed the lower values. From this time the growth rate increased but with a different trend. The fresh weight difference increased almost linearly while dry matter accumulation slowly increase until 63 daa, being constant until 150 daa and then increasing to stabilize at 240 dda. The same has been reported by Chao and Lovatt (2010) who found an increase of commercially valuable large-size fruit of "Fina Sodea" clementine mandarin using triclopyr.

The daily increase rate in all variables reached a maximum at 53 daa when the cell expansion growth of the juice sacs began, being higher in fruit of 2,4-D treated trees. From this time, both the fresh fruit weight as well as the dry fruit weight showed other peaks, but in all cases were higher in the 2,4-D treatment. Similar results concerning triclopyr treatment has been reported for mandarin (Agustí *et al.*, 2002; Roussos and Tassis, 2011).

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