REVISIÓN

CITRUS FRUIT QUALITY. PHYSIOLOGICAL BASIS AND TECHNIQUES OF IMPROVEMENT

Agustí, M.[1,] Martínez-Fuentes, A.[1], Mesejo, C.[1]

Recibido: 29/07/02 . Aceptado: 25/09/02

SUMMARY

Fruit size can be improved either by increasing carbohydrates availability to fruit or by increasing fruit sink strength. Application of synthetic auxins may act in these two ways, depending on the date of treatment. When they are applied during the physiological drop they have a thinning effect, reducing competition for carbohydrates among developing fruitlets; when applied at the onset of cell enlargement stage fruit sink strength is increased and carbohydrate accumulation in the fruit is enhanced.

Physiological disorders such as creasing, splitting, puffing, peel pitting, etc. can be reduced in intensity or minimized using gibberellic acid, synthetic auxins or a mixture of both. Fruit maturation (colouring) can be enhanced using ethephon or figaron and can be delayed using gibberellic acid. The effectiveness of some of these treatments can be significantly improved by adding mineral salts, mainly nitrogen compounds in the spray mix. In this article, best treatment strategies and their mechanism of action are discussed.

KEY WORDS: fruit size, physiological disorders, maturation, plant growth regulators.

RESUMEN

LA CALIDAD DE LOS FRUTOS CÍTRICOS. FUNDAMENTOS FISIOLÓGICOS Y TÉCNICAS DE MEJORA

El tamaño final de los frutos cítricos puede mejorarse bien aumentado la disponibilidad de carbohidratos bien aumentando la capacidad sumidero del fruto. La aplicación de auxinas de síntesis puede actuar en ambos sentidos, dependiendo de la época en que se apliquen. Durante la caída fisiológica de frutos aumentan la intensidad de ésta, reduciendo así la competencia por carbohidratos entre los frutos en desarrollo; cuando se aplican al inicio de la fase lineal del crecimiento del fruto aumentan su capacidad sumidero y la acumulación de carbohidratos en éste aumenta.

Algunos desórdenes fisiológicos, como la clareta, el rajado, el bufado, el picado, etc. pueden ser reducidos en intensidad o minimizados con la utilización de ácido giberélico, auxinas de síntesis o su mezcla. La maduración del fruto (coloración) puede adelantarse utilizando ethephon o figaron y puede retratarse con el uso del ácido giberélico. La eficacia de algunos de estos tratamientos puede mejorar significativamente mediante la adición de sales minerales, sobre todo nitrogenadas. En este artículo se discuten los tratamientos más eficaces y su mecanismo de acción.

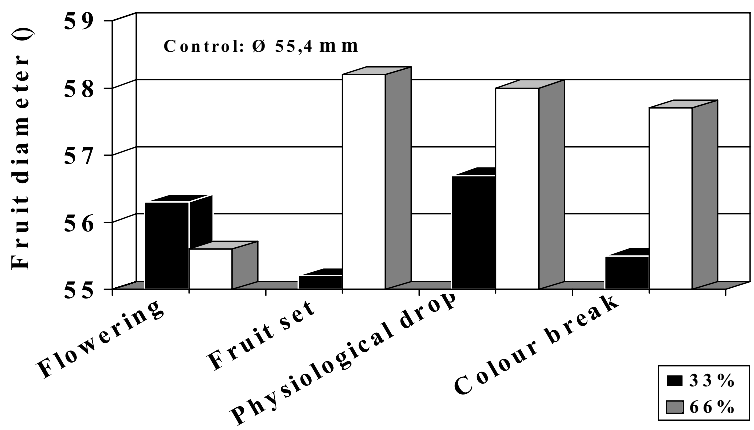
PALABRAS CLAVE: tamaño del fruto, alteraciones fisiológicas, maduración, reguladores del desarrollo.

[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica, Camino de Vera, s/n, 46022 Valencia, Spain. magusti@prv.upv.es

FRUIT SIZE

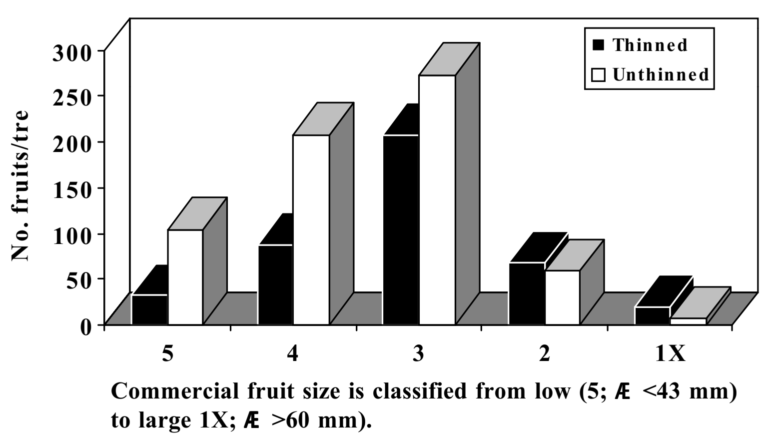
Fruit size represents one of the most important factor of quality for citrus fresh consumption. Factors controlling citrus fruit size have been thoroughly studied (Goldschmidt and Monselise, 1977; El-Otmani *et al.,* 1995; 2000; Agustí and Almela, 1991; Agustí, 1999; Guardiola and García-Luis, 2000) and there are several techniques with a capacity of size improvement, such as girdling (Cohen, 1984), the application of fruit thinner agents (Wheaton, 1981; Gallasch, 1988; Agustí *et al.,* 1996) and the application of fruit enhancers substances (Agustí and Almela, 1991; Agustí *et al.,* 1994; 1995; Aznar *et al.,* 1995; El-Otmani *et al.,* 1993).

Synthetic auxins have been widely used as thinner agents in citrus. The mechanism of action of these substances has been suggested through an increase of the sink capacity of the leaves that, in turn, reduces transport of metabolites to the fruit (Iwahori, 1978) and their effectiveness depends on the auxin type, formula, concentration applied and date of treatment (Ortolá *et al.,* 1991; Agustí *et al.,* 1996). But a significant increase in fruit size occurs only if fruit thinning is higher than 50-60% of total fruits and if it is performed early in fruit development; it has been demonstrated both manually (Fig. 1; Zaragoza *et al.,* 1992) and chemically (Fig. 2; Agustí *et al.,* 1995a; 1995b).



Source: Zaragoza *et al.*, 1992.

Fig. 1. Effect of intensity (33% and 66%) and date of thinning on final fruit size of 'Clausellina' Satsuma mandarin fruit.



Source: Agustí *et al.*, 1995a.

Fig. 2. Effect of chemical thinning on the distribution of commercial fruit calibres of 'Owari' Satsuma mandarin. Treatment with naphthalene acetic acid (500 mg l^{-1}) applied during the physiological fruit drop. No. of fruits/tree: Unthinned trees: 651; thinned trees: 416 (thinning intensity: 37%).

Experiments with synthetic auxins have allowed separating between thinning effect and fruit size enhancer effect by modifying the date of treatment (Agustí *et al.,* 1995b; Fig. 3). The application at the onset of cell enlargement stage increases significantly the final fruit size with no thinning effect (Agustí *et al.,* 1994). At this stage, applying auxin stimulates cell expansion, especially of juice vesicles (Table 1; El-Otmani *et al.,* 1993; Agustí *et al.,* 1995b;

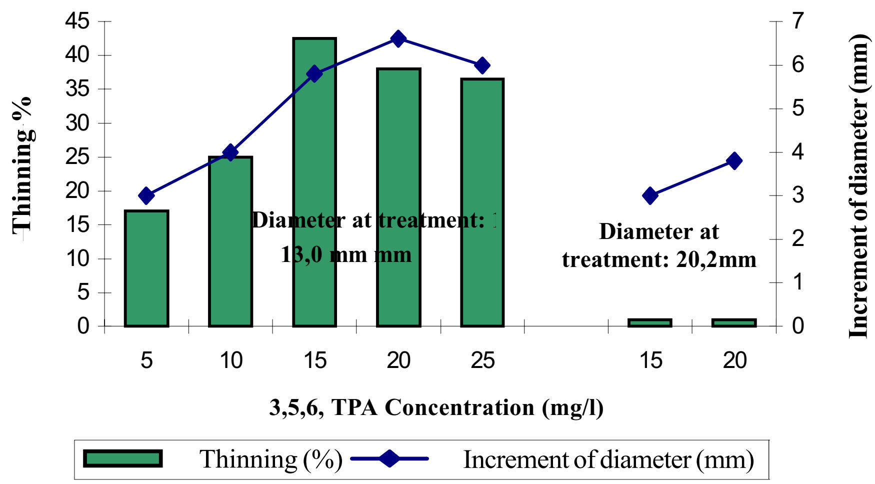


Fig. 3. Influence of the concentration and the date of application of 3,5,6 TPA on thinning and diameter increment of 'Clementina fina' mandarin fruit.

Table 1. Effect of 2,4 DP applied at the onset of cell enlargement stage on the characteristics of rind, locules and juice vesicles of 'Fortune' mandarin fruit[z].

Characteristic	2,4-DP (mg l^{-1})		
	0	50	Significance
Fruit diameter (mm)	22.0	26.7	5%
Peel weight (g)	20.0	29.7	5%
Peel thickness (mm)	4.0	4.0	n.s.
Locule width			
Radial (mm)	5.3	7.2	5%
Tangencial	2.9	4.0	5%
Vesicle/locule			
Radial width	4.0	4.2	n.s.
Tangential width	5.6	6.0	n.s.
Vesicles			
Length (mm)	1.31	1.75	5%
Width (mm)	0.52	0.62	5%
Fresh weight (mg)	26.1	42.3	5%
Dry weight (mg)	3.66	4.65	5%
Juice/vesicle			
mg	22.5	37.6	5%
% (w/w)	86.0	89.0	n.s.
Vesicle epidermal cells			
Radial width (µm)	33.33	54.80	5%
Tangential width (µm)	21.81	29.10	5%
Rows of cells per vesicle	24.06	24.83	n.s.

[z] Peel weight and thickness, vesicle weight and juice content are from mature fruits, the other parameters are of fruits sampled 20 days after treatment. n.s.: Not significant.
Adapted from El-Otmani *et al.,* 1993.

Aznar *et al.,* 1995). This cell expansion increases vesicle capacity for juice accumulation and, therefore, the fruit grows faster. Delaying treatment, until cell expansion diminishes or ceases, makes treatment less effective (Fig. 4) (Agustí *et al.,* 1994; Guardiola and Lázaro, 1987).

The activation of cell expansion by auxins requires a maintained cell turgor (Cleland, 1987), which is caused by photosynthates synthesized actively by the leaves in response to auxin treatment. Thus, the application of the synthetic auxin 3,5,6-TPA at the cell enlargement stage increases hexoses in developing fruit and although the sucrose concentration also increases, treatment reduces sucrose/hexose ratio compared with control fruit, suggesting that sucrose is more rapidly metabolized in treating developing fruit (Agustí *et al.,* 2002). The results suggest that the enhancer effect of synthetic auxins on fruit growth operates via promotion of sink strength.

Nowadays synthetic auxins are widely used as fruit size enhancers in the main countries producing mandarins for fresh consumption; 2,4-DP (Fig. 4) and 3,5,6-TPA (Fig. 3) have shown to have a powerful effect increasing the final fruit size with increments for Clementine mandarins by between 3-4 mm and 4-6 mm in diameter, respectively, and provoking a significant shift of commercial fruit size distribution to the larger sizes (Fig. 5) (Agustí *et al.,* 1996). Fruit characteristics are not modified by their application, except those related to fruit size (i.e. peel weight, juice content, etc.) that are increased in absolute value, but not in relative value to fruit weight (El-Otmani *et al.,* 1993; Agustí *et al.,* 1994).

Girdling or ringing also can be used to increase final fruit size. Girdling and ringing differ in their width of the girdle; girdling consists of removal a ring of bark from scaffold branches, whereas ringing consists of a single cut of about 1 mm width on the branch around its circumference, without removing bark. In fruit trees, there are evidences that the magnitude of the response does not depend on the width of girdled (Agustí *et al.,* 1998); moreover, tree injury caused by ringing is petty compared with girdling and neals quicker than girdling. Application date significantly affects the response since when it is carried out at early stages of fruit development significantly

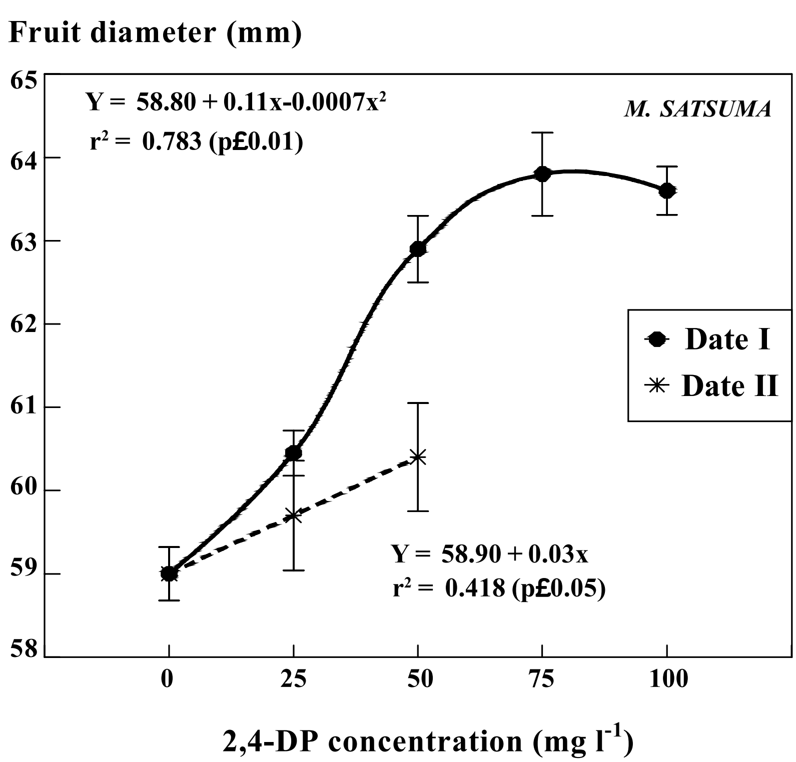


Fig. 4. Influence of 2,4-DP concentration and treatment date on fruit size of Òwari Satsuma mandarin at maturity. Fruit diameter at treatment on Date I was 25.1±0.4 mm, and on Date II it was 30.7±0.3 mm.

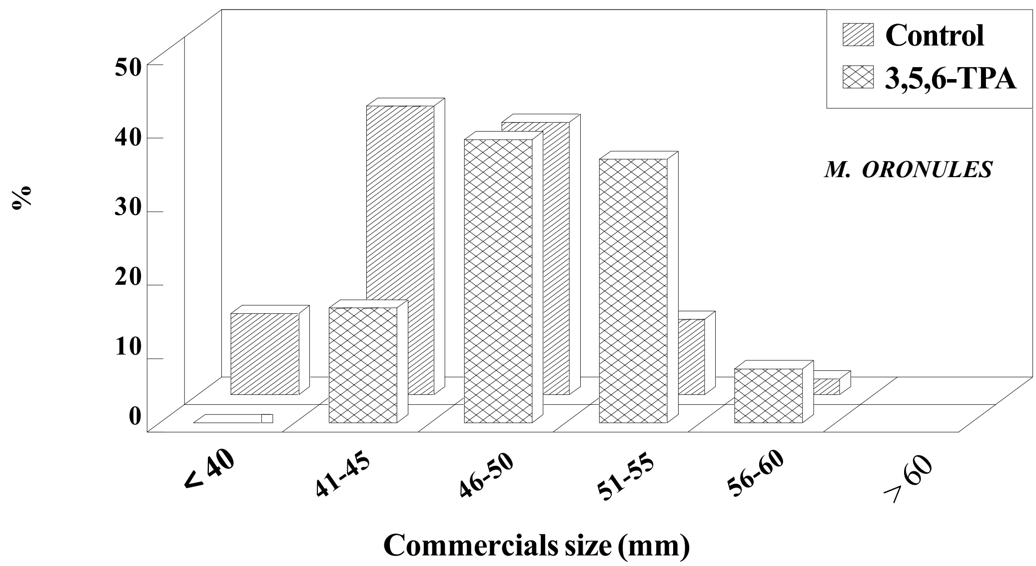
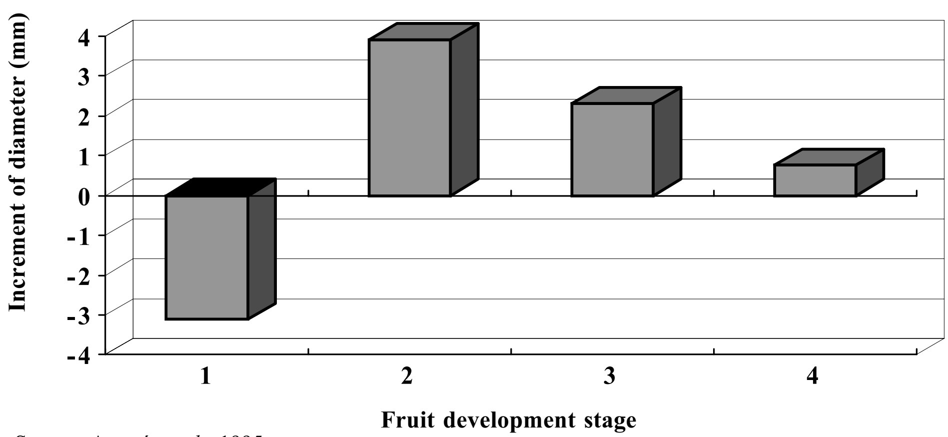


Fig. 5. Influence of 3,5,6-TPA on frequency distribution of 'Oronules' Clementine mandarin fruit diameters at harvest. Average values of treated fruit correspond to 3,5,6-TPA at 0 and 10 mg l^{-1} applied at the end of physiological fruit drop.

increased fruit set and, consequently, it reduces fruit size. But when girdling is performed at the onset of cell enlargement stage, increases significantly the final fruit size and improves commercial fruit size distribution in a similar intensity to that of synthetic auxins (Fig. 6) (Agustí *et al.,* 1995a); delaying treatment reduces the effectiveness. As for treatments with synthetic auxins, fruit characteristics are not modified by ringing, except those related to fruit size.



Source: Agustí *et al.*, 1995a.

Fig. 6. Effect of ringing date on fruit size of 'Owari Satsuma' mandarin at maturity. Date of ringing: 1. Physiological fruit drop; 2. The onset of cell enlargement stage; 3. Cell enlargement stage; 4. Fruit colour break.

Girdling has been shown to cause accumulation of carbohydrates in tree organs above the girdle; accordingly, its effects are brought about by the increased availability of carbohydrates (Goldschmidt and Koch, 1996).

PHYSIOLOGICAL DISORDERS

Splitting

Splitting is a physiological fruit disorder manifested as a meridian fissure of the peel, usually developing from the stylar end and reaching, or even extending beyond, the equatorial zone. Splitting is a frequent problem in oranges and mandarins all over the word (Erickson, 1957; Lima *et al.,* 1980; Bar Akiva, 1975; De Cicco *et al.,* 1988; Ruiz and Primo-Millo, 1989).

The causes of fruit splitting are not well understood, although seasonal water deficits followed by rains during cell enlargement fruit development stage, have been closely related with the number of 'Nova' mandarin affected fruits (Almela *et al.,* 1990); however, rainfall have not been correlated with splitting for 'Ellendale' mandarin (E. Rabe cited by Davies and Albrigo, 1994).

Irrespective to its origin, splitting develops as a consequence of disruption between peel and pulp growth. During the phase of cell enlargement, if the peel does not re-start its growth when pulp expansion takes place, the fruit splits (Erner *et al.,* 1975). Although the albedo may alleviate pulp pressure because of its sponginess, the flavedo tissues are more rigid and will eventually crack (Kaufman, 1970). This appears to be the reason for the negative correlation found between peel thickness or peel resistance to puncturing and the number of fruits affected by splitting (Cohen *et al.,* 1972; Almela *et al.,* 1994).

The application of calcium nitrate sprays at the beginning of cell enlargement stage significantly reduces the proportion of fruit affected by splitting (Monselise and Costo, 1985), but the response is often erratic (Almela *et al.,* 1994). Best results are obtained with the application of a mixture of gibberellic acid and 2,4-dichlorophenoxyacetic acid (Monselise and Costo, 1985); treatment significantly reduces fruit splitting and the repetition of the treatment improves the response (Fig. 7). Treatments do not increase peel thickness but significantly increase peel resistance to puncturing; the substances applied probably act on fruit splitting through an increase in peel resistance (Almela *et al.,* 1994).

Cold pitting

Cold pitting or peel pitting is a physiological disorder usually related to post-harvest storage conditions (Pantastico *et al.,* 1968; Cohen *et al.,* 1990; 1994), but in some cases, as for 'Fortune' mandarin, peel-pitting appears before harvesting (Almela *et al.,* 1992).

Pre-harvest peel pitting starts on fruit as discrete areas forming sunken reddish-brown to black lesions that tend to coalesce producing larger depressions of affected areas

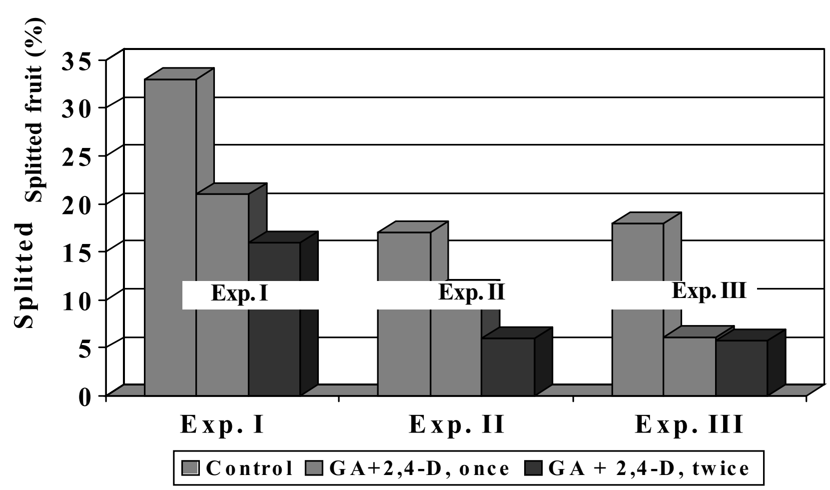


Fig. 7. Effect of GA_3 (20 mg l^{-1}) and 2,4 D (20 mg l^{-1}) applied as a mixture, on the incidence of fruit splitting of 'Nova' mandarin. Treatments applied after june drop. Values corresponding to three experiments in different years.

(Almela *et al.,* 1992). The incidence of this disorder varies from year to year and even among fruits of a given tree.

The cause of pre-harvest peel-pitting is not well known, although cold and dry winds and both low temperature and relative humidity have been suggested as responsible for pitting (Pantastico *et al.,* 1968; Almela *et al.,* 1992; Cohen *et al.,* 1994). These climatic conditions change the physiological properties of membranes and cuticles (Vercher *et al.,* 1994) and modify the water balance of injured areas.

Breakdown of epidermal cells is the first event of peel-pitting. It has been related to tonoplast disruption and to the concomitant accumulation of phenolic substances (Sawamura *et al.,* 1984) and volatiles under the cuticle, such as acetaldehyde, ethanol and ethylene (Cohen *et al.,* 1990), which are, apparently, responsible for development of pitting (Abe, 1990). The shape of the injured epidermal and hypodermal cells are responsible for undulating and depressed appearance of the rind in cross-section (Vercher *et al.,* 1994).

The depressed areas in affected fruit are devoid of crystalline wax structures and have crushed epidermal and hypodermic cells with unfolded walls; despite of it no sign of disruption in the cuticle is observed (Vercher *et al.,* 1994). These cells are either empty or filled with reduced cytoplasm amounts located in central position of the cell (Fig. 8) (Vercher *et al.,* 1994).

The application of calcium nitrate just before or at fruit color break has been shown to be effective controlling peel-pitting of 'Fortune' mandarin in Spain. In our experiments, the percentage of affected fruit was significantly reduced by between 33% and 81% (Zaragoza *et al.,* 1996; Table 2). The loss of calcium from the cell walls during ripening causes solubilization of pectin (Doesburg, 1975) and accelerates senescence; on the other hand, the concentration of calcium in the albedo tissues of navel orange decreases during on-tree storage (Lewis *et al.,* 1967) and there are evidences of a relationship between the reduction of peel-pitting of 'Fortune' mandarin and the decrease of water permeability associated with the use of calcium nitrate (Zaragoza *et al.,* 1996). Gibberellic acid also reduces the percentage of affected fruits of 'Fortune' mandarin, although with a lower efficiency (Zaragoza *et al.,* 1996). Its effectiveness is due to its control of peel senescence through a reduction of wax accumulation on the fruit surface (El Otmani and Coggins, 1985), consequently senescence seems not to be the unique factor involved in this physiological disorder.

Some antitranspirants, such as pinolene, a polyterpene film former, replaces the loss of crystalline waxes and, in this way, can substitute it for their action, reducing the water loss of fruit. Agustí *et al.* (1997) reduced the percentage of peel-pitted fruits of 'Fortune' mandarin by between up to 70% by spraying of pinolene at a concentration of 0.7% at fruit color break.

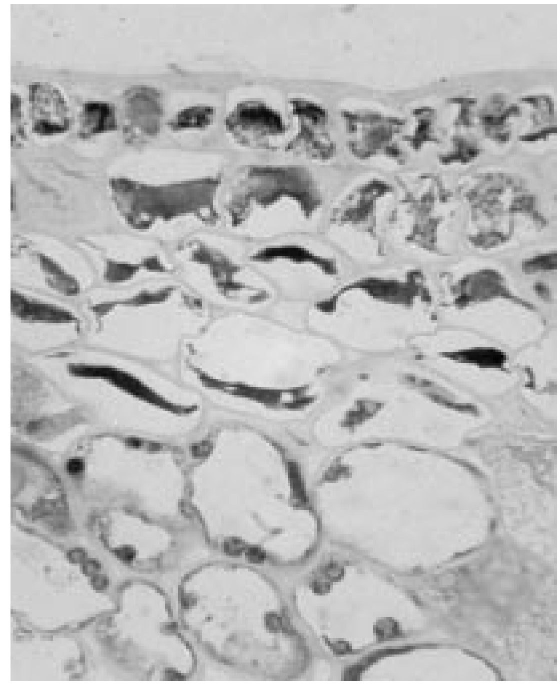


Fig. 8. Cross-section of rind from ‘Fortune’ mandarin fruit with moderate symptoms of *peel pitting* showing a collapsed cytoplasm located in central position of the cell. Section was stained with ruthenium red. *From*: Vercher *et al.,* 1994.

Puffing

Puffing is a physiological disorder characterized by separation between peel and pulp. It is related to the disintegration of the deepest cell layers of the albedo tissue that gives rise to aerial spaces (Kuraoka, 1962). The development of these spaces results in a cracked and low resistant albedo of mature fruits. The symptoms increase as peel grows just after the pulp has completed its development (García-Luis *et al.,* 1985). This belated peel growth takes place only in a few mandarin varieties, such as Satsuma mandarin (Kuraoka, 1962) or 'Oroval' clementine mandarin, which are inclined to puffing.

The cause of puffing has been related to the water exchange regulation through the peel (Kawase *et al.,* 1981). Accordingly, high values of RH together with high temperatures at fruit color-break increase the appearance and intensity of puffing, particularly after a period of drought.

Table 2. Effectiveness of calcium nitrate [$Ca(NO_3)_2.4H_2O$], GA_3 and their mixture on the percentage of 'Fortune' mandarin fruits affected by peel-pitting.

Expt. Nº.	Control	$Ca(NO_3)_2.4H_2O$	GA_3	$Ca(NO_3)_2.4H_2O + GA_3$	Signif.
I	43 b	20 a	29 ab	-	5%
II	67 b	45 a	-	-	5%
III	63 b	-	46 a	-	5%
IV	60 b	23 a	48 b	38 ab	5%
V	13 c	5 a	10 b	11 b	5%
VI	31 b	6 a	-	-	1%
VII	69 b	38 a	-	54 ab	5%

Source: Zaragoza *et al.*, 1996.

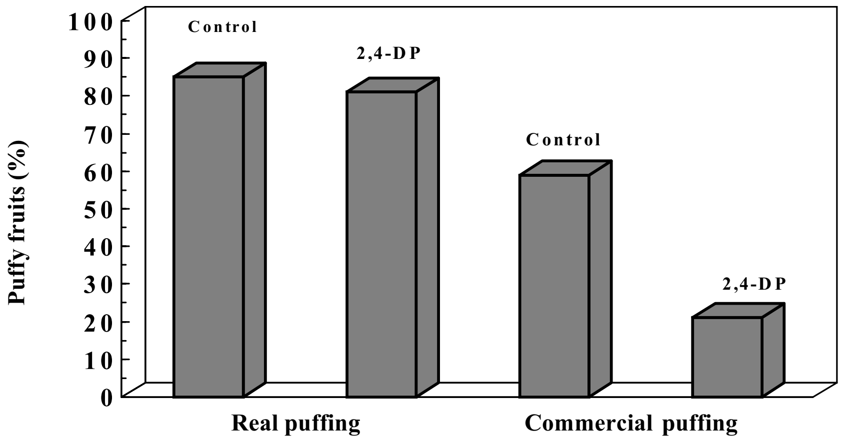
The application of 10 mg l^{-1} of GA_3 before fruit color break reduces the occurrence of puffing in Satsuma mandarin (Kuraoka *et al.,* 1966; Agustí *et al.,* 1981). The GA_3 treatment prevents the late growth of the peel and increases the compactness of the albedo (García-Luis *et al.,* 1985). The addition of nitrogen compounds reinforces the effect of GA_3 (Agustí *et al.,* 1981). A reduction of percentage of puffy fruit from 56% to 18% has been obtained on Satsuma mandarin applying a mixture of GA_3 (10 mg l^{-1}) and ammonium nitrate (1.8%) 30 days before fruit color break (Table 3). The application of synthetic auxins (e.g. 2,4-DP) at the onset of cell enlargement stage to increase fruit size also controls fruit puffing (Agustí *et al.,* 1994); the peel of treated fruit is more resistant and even puffing is not prevented under the physiological point of view, its commercial incidence at harvest is significantly reduced by more than 80% (Fig. 9)

The main internal fruit characteristics are not modified by treatments, except those related to peel characteristics that are significantly altered. At the concentration mentioned above, GA_3 prevents the increase of the peel weight, increases its resistance to puncturing and reduces its rate of chlorophyll loss. This relationship found between the GA_3 effect on chlorophyll retention in the rind and the prevention of puffiness, together with its effect on increasing the compactness of the albedo, allows to conclude that puffiness is determined by the viability of the flavedo cell layers (García-Luis *et al.,* 1985).

Table 3. Effect of GA_3 (10 mgl-1) applied alone or in combination with nitrogen compounds on the percentage of 'Satsuma' mandarin fruits affected by *puffing.*

Nitrogen compound	$-GA_3$	$+ GA_3$
	56.3^z	32.7 a
Ammonium phosphate (1.5%)	50.5	25.7 ab
Potassium phosphate (2.7%)	51.1	34.7 a
Ammonium nitrate (1.8%)	66.4	18.1 b
Potassium nitrate (2.0%)	50.3	27.7 a
Magnesium nitrate (1.5%)	50.0	30.7 a
Urea 0.8%)	59.8	27.6 a
Significance	---	5%

^{z} $+GA_3$ significantly differs from $-GA_3$ (p£0.05).
Source: Agustí *et al.*, 1986.



Date of evaluation: January 16.
Source: Agustí *et al.*, 1994.

Fig. 9. Effect of 2,4-DP (50 mg l^{-1}) applied at the onset of cell enlargement stage on the control of puffing of 'Satsuma' mandarin.

Creasing

Creasing is a physiological disorder of fruits causing cracks in cell layers of albedo tissue of peel; it corresponds with depressions on the rind that alternates with healthy areas that turn bulky (Jones *et al.,* 1967). According to Storey and Treeby (1994), albedo cells are tubular in shape and those lining the cracks separated at the middle lamella leaving like-protruding stumps; this separation sometimes takes place with no damage to the cells so that the cells retain their turgor, but many cells are irreparable damaged, lose their turgor and wall collapses.

The cause of creasing is not yet clearly understood. Climatic factors, cultural practices and endogenous factors have been related with this physiological disorder (Hotzhausen, 1981).

Creasing has been connected with pectin degradation, loosening the connection among the cells of the albedo tissue. Promotion of pectinmethylesterase activity of albedo tissue of creased fruit was demonstrated by Monselise *et al.,* (1976), who also found a significant increase on the amount of water-soluble pectins. Both factors increase in over-ripe fruits, explaining why albedo cells become large and lobe in senescent fruits and why the authors suggested that albedo breakdown is the result of an early onset of senescence. However, Storey and Treeby (1994) disagree with this hypothesis and demonstrated that albedo cells from creased tissue are relatively small; this authors suggest that changes in the cohesion of neighbouring cells at the middle lamella predisposes the fruit to creasing.

The application of GA_3 (10-20 mg l^{-1}) at early stages of fruit development (Æ 30-40 mm) or just prior to fruit colour break, reduces considerably the incidence of creasing (Monselise *et al.,* 1976). As for puffiness, the addition of nitrogen compounds reinforces its effect (Ruiz and Primo-Millo, 1989; Table 4). It had a strong inhibition effect on color development when applied close to colour-break. Albedo cells division or active growth are the main periods when GA_3 is effective in reducing creasing (Monselise *et al.,* 1976).

Gibberellins induce long-term formative effects in albedo tissue, delay their senescence (Lewis *et al.,* 1967) and increase its compactness making it less prone to creasing. This situation contrasts with the effect of GA_3 on puffing, where GA_3 application at earlier stages of fruit growth does not control the alteration, although the treatment also increases the compactness of the albedo (García-Luis *et al.,* 1985).

Peel disorders linked to fruit senescence

In citricultures for fresh consumption, an oversupply often occurs, with a consequent fall in price. In Clementine mandarins, it is of the utmost importance to extend their picking season. But the on-tree storage of fruit up to its plain maturity leads to the appearance of physiological disorders linked to peel senescence, such as discolouration, stains, and blemishes, that diminish fruit quality. After fruit color break, high temperature and high relative humidity accelerate the process.

Table 4. Effect of gibberellic acid (12 mgl^{-1}), potassium nitrate (2%) and ammonium phosphate (2%), applied alone or as a mixture, , on the proportion of 'Valencia' fruits affected by *creasing*. Influence of date of treatment. Source: Ruiz and Primo-Millo, 1989.

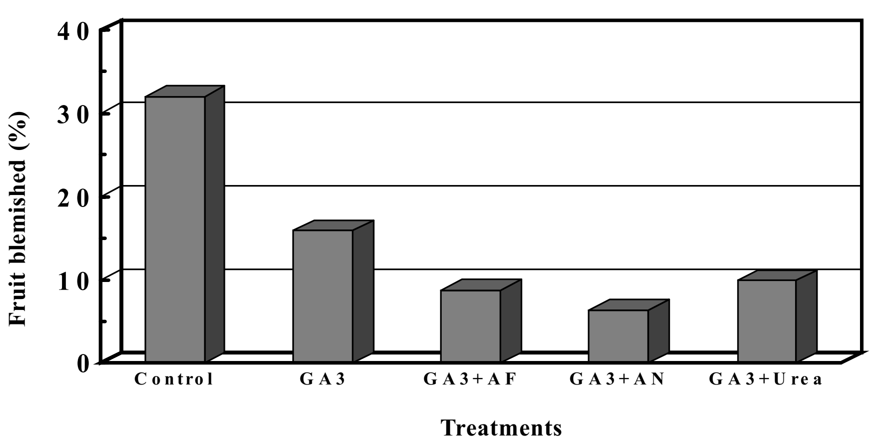
Treatment	Affected fruit (%)
Control	58.7 a
GA_3, July	11.3 b
GA_3, September	14.1 bc
GA_3, November	27.4 c
$GA_3 + KNO_3$, July	5.6 d
$GA_3 + (NH4)_2HPO_4$, July	7.7 bd
KNO_3, July	33.9 c
$(NH4)_2HPO_4$, July	44.5 e

Some of these disorders can be partially controlled by applying gibberellins (Coggins and Eaks, 1964; Guardiola *et al.,* 1981). The application of 5 mg l^{-1} of GA_3 once and prior to fruit color break, is enough to delay the peel senescence for more than 30 days with a consequent delay on the appearance of peel disorders linked to the process (Fig. 10). As for other physiological disorders related to peel tissues feasibility, nitrogen compounds enhance the effectiveness of GA_3. Treatments do not affect internal fruit characteristics, which is particularly important for Clementine mandarins; this group of mandarins loses the juice progressively after fruit color break and GA_3 treatment does not stop it; consequently, 30-50 days after treatment it is possible to pick fruits of high external quality but with very low juice content (Fig. 11).

Fruit abscission

Fruit of navel oranges and of some hybrids are prone to abscission as soon as they overcome the maturation process. Applying synthetic auxins can efficiently control it. The application of 2,4-D (15 mg l^{-1}) delays significantly fruit abscission of navel oranges (Stewart and Klotz, 1947). Treatments must be carried out prior to the abscission process. Those applied one month before color break have shown efficacious retarding fruit abscission of 'Navelate' sweet orange more than 5 months (Table 5). Repetition of treatment two months later has no additional effect except if more than 5 months of on tree storage is requested. Other synthetic auxins, such us 2,4,5-TP (Coggins and Hield, 1968) and 3,5,6-TPA (Almela *et al.,* 1997; Table 6), have shown as effective as 2,4-D.

Internal fruit characteristics are not altered by treatments; however, since treatments are applied to delay harvesting, rind disorders associated to senescence must be prevented by adding GA_3. The delay of harvest time reduces the intensity of the next flowering and it must be taken into account, especially in the case of alternate bearing varieties.



Adapted from Agustí *et al.*, 1988.

Fig. 10. Effectiveness of GA_3 (10 mg l^{-1}) and simultaneous application of ammonium compounds controlling rind blemishes associated to senescence of 'Clemenules' mandarin fruit (AF: Ammonium phosphate; 1,5%; AN: Ammonium nitrate; 1,8%; Urea; 0,8%).

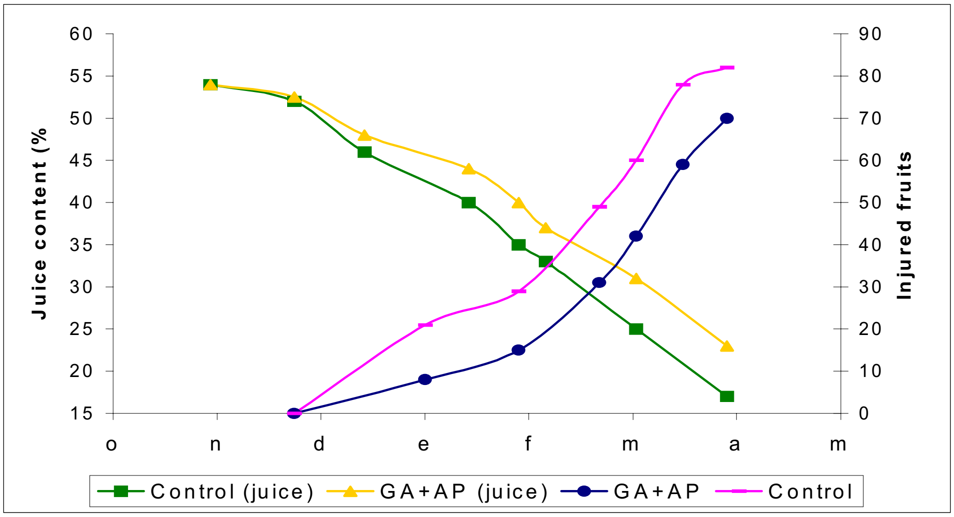


Fig. 11. Effect of the application of gibberellic acid (10 mgl^{-1}) (GA) and biammonium phosphate (1,5%) (AP) on the evolution of the alterations associated to senescence and juice content of 'Clemenules' mandarin.

Table 5. Effectiveness of gibberellic acid (10 mgl^{-1}) and 2,4-D (15 mgl^{-1}) on the control of fruit abscission of 'Navelate' sweet orange. Influence of date treatment

Date of treatment	Abscission (%)		Calix resistance to pull off (kg)	
	March, 23rd	May, 31st	March, 23rd	May, 31st
Control	31.8 a	70.2 a	2.2 a	3.7 a
Oct., 30th	5.9 b	15.6 b	5.9 b	4.4 b
Nov., 26th	2.4 b	18.7 b	6.5 b	4.7 b
Dec., 22nd	5.5 b	14.1 b	6.1 b	5.2 b
Oct + Dec^{z}	5.1 b	7.9 b	7.6 b	5.2 b
Oct + Dec^{y}	2.3 b	7.0 b	7.4 b	5.3 b
Significance	5%	5%	5%	5%

^{z} 2,4-D applied in December: 7.5 mgl^{-1}
^{y} 2,4 D applied in December: 15 mgl^{-1}
Source: Almela *et al.,* 1997.

Navel rind stain

Fruits of Navel oranges can be severely affected by the physiological disorder known as rind stain or rind breakdown. Initially, injured fruits show small depression on the rind with no changes in color and with oil glands remaining intact. The disorder begins at the flavedo-albedo union area, which cells become dehydrated and flattened, and finally die. Despite of it, cuticle did not shown any sign of disruption or damage. When damage riches up the flavedo and epidermis, their cells die as well and develop brown to black lesions of necrotic depressed areas (Agustí *et al.,* 2001).

The cause of this physiological disorder has been related to nutritional imbalances, drought and rainy periods in alternation (Zaragoza and Alonso, 1975) and cold periods

Table 6. Effect of 2,4-D and 3,5,6-TPA on fruit abscission of 'Navelina' sweet orange. Date of treatments: Nov., 30th.

Treatment	Abscission (%)		Calix resistance to pull off (kg)	
	Feb., 1st	March, 23rd	Feb., 1st	March, 23rd
Control	9.9 c	20.1c	6.5 a	2.3 a
2,4 D; 15 mg l^{-1}	1.4 b	4.9 b	7.5 b	3.9 b
3,5,6 TPA, 7.5 mg l^{-1}	0.9 b	4.3 ab	6.9 ab	3.4 b
3,5,6 TPA, 15 mg l^{-1}	0.0 a	3.1 ab	7.7 b	4.1 b
3,5,6 TPA, 22.5 mg l^{-1}	0.0 a	1.5 a	7.8 b	5.0 c
Significance	5%	5%	5%	5%

Source: Almela *et al.*, 1997.

(Klotz *et al.,* 1966). The incidence of navel rind stain varies in intensity from year to year, among orchards and even among varieties, affecting up to 50% of mature fruits in some cases, such as 'Navelate' in Spain. Fruit position on the tree has shown as important factor of developing rind breakdown, fruits outside of canopy being most sensitive fruits and the outside face of fruit being more sensitive than inside face (Almela *et al.,* 2000). In 'Navelate' oranges stored at 20ºC, transference of fruit from low (45%) to high (95%) RH agravates the incidence of the disorder (Zacarías *et al.,* 2001).

Nowadays we have not effective treatments to control it. However, rootstock plays an important influence in the development of the disorder citrange.

Carrizo citrange is proner to give the disorder to the variety than Cleopatra mandarin, and it, in turn, proner than sour orange (Table 7). This dependence has been related to rootstock influence on water transpiration capacity, supported by the histological study of fruit peduncle (Almela *et al.,* 2000).

Fruit maturation

Delay or advance fruit maturation is of great interest in the mandarin-producing countries for fresh market since it allows to extend the period of commercialization.

Fruit color break can be advanced by means of the use of ethylene releasing compounds. Spraying 100 to 200 mg l^{-1} ethephon accelerate colouration and thus harvest of mandarins by 1 to 3 weeks (Iwahori, 1978; Pons *et al.,* 1992); this effect can be enhanced by combining the treatment with a degreening in chamber with ethylene (Pons *et al.,* 1992; Fig. 12). Its effectiveness depends on the date of treatment, the best results being obtained for 20-25 days before the usual date of color break (Pons *et*

Table 7. Influence of rootstock on the proportion of 'Navelate' sweet orange affected by rind breakdown. Values expressed as percentage of affected fruits per tree.

Year	Carrizo citrange	Cleopatra mandarin
95/96	65.8 ± 1.4	54.9 ± 5.2
96/97	28.9 ± 3.3	18.0 ± 2.9
97/98	65.6 ± 3.2	21.5 ± 2.9
98/99	63.3 ± 4.9	47.8 ± 4.6
99/00	82.0 ± 3.5	82.8 ± 3.2
	Carrizo citrange	**Sour orange**
98/99	62.0 ± 3.1	9.0 ± 0.5
	82.6 ± 5.0	6.0 ± 0.6
99/00	65.8 ± 5.6	24.1 ± 4.8

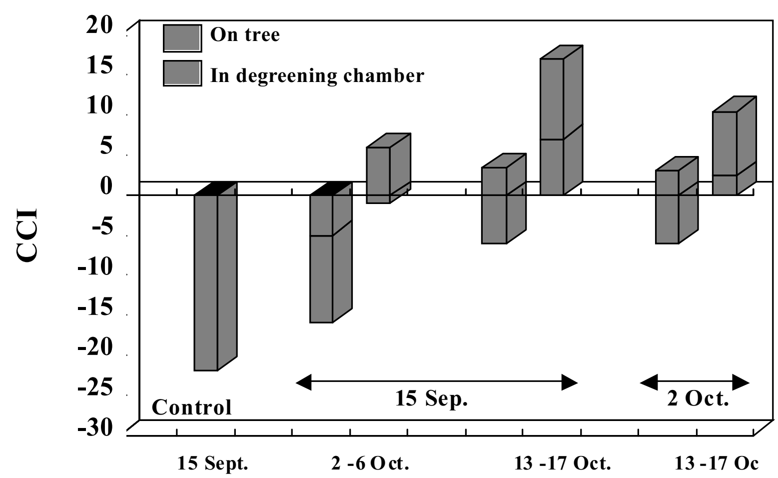
Source: Almela *et al.*, 2000.

al., 1992). By spraying 200 mg l^{-1} ethephon or higher concentration, an important leaf and fruit abscission may occur. Although these negative effects are closely related to temperature at treatment time, the mixture of calcium salts, both nitrate and acetate, at 4.5 g l^{-1} of calcium, reduces considerably leaf and fruit abscission, but also reduces the effectiveness of ethephon accelerating fruit coloration (Pons *et al.,* 1992). Ethephon does not change the internal quality of the fruit in terms of total soluble solids and titratable acidity (Iwahori, 1978; Pons *et al.,* 1992).

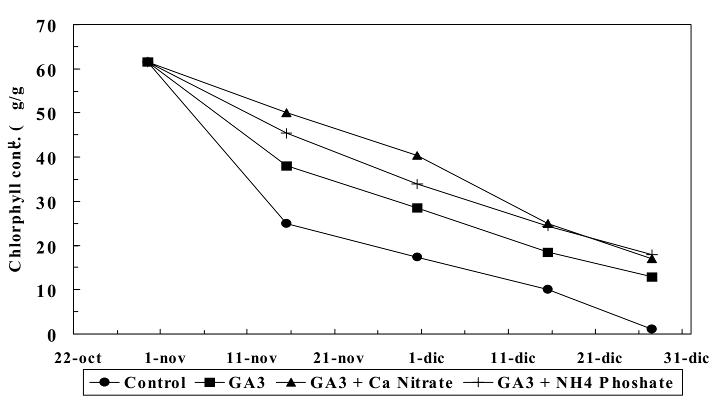
Ethylchlozate also accelerates fruit colouration of mandarins (Kamuro and Hirai, 1981) and modifies internal characteristics of the fruit. Application of 100-200 mg l^{-1} of ethylchlozate significantly increased Brix and citric acid content of 'Ponkan' mandarin in Japan (Iwahori *et al.,* 1986). In Spain, same concentrations also increased Brix, but did not affect citric acid content of 'Clausellina' (Casas and Mallent, 1983) and 'Owari Satsuma' and 'Oroval' and 'Marisol' Clementine mandarins (Pons *et al.,* 1988; Liegeois *et al.,* 1995). Although time of application does not seem to be critical, best results are obtained by two applications at 15-day intervals between 80 and 110 days after full bloom.

Delay peel colouring is easily to obtain by treating fruits with GA_3 prior to color break (see control of puffing, creasing and peel disorders linked to fruit senescence). Ten to twenty mg l^{-1} allows to retard fruit colouring by between 30 to 45 days and nitrogen compounds reinforces the action (Fig. 13) (Jackson *et al.,* 1992). Delay internal maturation is not feasible today, at least under a commercial point of view.

Fig. 12. Effect of Ethephon (200 mgl^{-1}) on the evolution of Citrus Colour Index (CCI[z]) in the peel of Clementine mandarin, cv. 'Marisol'. Influence of date of treatment (15 Sept. and 2 Oct.).



[z] See CCI in Jiménez-Cuesta *et al.*, 1981.
Adapted from Pons *et al.*, 1992.



GA_3: 10 mg l^{-1}; Ca Nitrate: 2%; NH_4 Phosphate: 1,5%
Source: Jackson *et al.*, 1992.

Fig. 13. Changes in chlorophyll concentration in the peel of 'Fortune' mandarin. Effect of gibberellic acid and its mixture with nitrogen compounds. Treatments applied on 31 october.

LITERATURE

ABE, K. 1990. Ultrastructural changes during chilling stress. p. 71-84. In: C.Y. Wang (ed.). *Chilling injury of horticultural crops,* CRC Press, Inc., Boca Raton, Florida, USA.

AGUSTÍ, M. 1999. Preharvest factors affecting postharvest quality of citrus fruit. p 1-34. In: M. Schirra (ed.). *Advances in postharvest diseases and disorders control of citrus fruit*., Research Signpost, Trivandrum, India, pp 1-34.

AGUSTÍ, M., ALMELA,V. 1991. *Aplicación de fitorreguladores en citricultura*. Ed. AEDOS, Barcelona, Spain.

AGUSTÍ M., ALMELA, V., AZNAR, M., EL OTMANI, M., PONS, J. 1994. Satsuma mandarin fruit size increased by 2,4 DP. HortScience 29:279 281.

AGUSTÍ, M., ALMELA, V.,GUARDIOLA, J. L. 1981. The regulation of fruit cropping in mandarins through the use of growth regulators. Proc. Int. Soc. Citriculture, 216-220.

AGUSTÍ, M., ALMELA, V., GUARDIOLA, J. L. 1986. Recolección tardía del fruto en el mandarino Satsuma. Efecto del ácido giberélico. Actas II Congreso SECH: 352-360.

AGUSTÍ, M., ALMELA, V., GUARDIOLA, J. L. 1988. Aplicación de ácido giberélico para el control de alteraciones de la corteza de las mandarinas asociadas a la maduración. Invest. Agr. Prod. Prot. Veg., 3: 125-137.

AGUSTÍ, M., ALMELA, V., ZARAGOZA, S., PRIMO MILLO, E., EL OTMANI, M. 1996. Recent findings on the mechanism of action of the synthetic auxins used to improve fruit size of citrus. Proc. Int. Soc. Citriculture, 2:922-928.

AGUSTÍ, M., ALMELA, V., ZARAGOZA, S., GAZZOLA, R., PRIMO-MILLO, E. 1997. Alleviation of peel-pitting of 'Fortune' mandarin by the polyterpene pinolene. J. Hort. Sci., 72: 653-658.

AGUSTÍ, M., ANDREU, I., JUAN, M., ALMELA, V., ZACARÍAS, L. 1998. Effects of ringing branches on fruti size and maturity of peach and nectarine cultivars. J. Hort. Sci. & Biotechnol., 73: 537-540.

AGUSTÍ, M., ALMELA, V., AZNAR, JUAN, M., V. ERES, V. 1995a. *Desarrollo y tamaño final del fruto en los agrios*. Generalitat Valenciana, Serie Divulgació Técnica n. 32.

AGUSTÍ, M., ALMELA, V., JUAN, V., ALFÉREZ, F., TADEO, F.R., ZACARÍAS, L. 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. Bot., 88: 415-422.

AGUSTÍ, M., EL OTMANI, M., AZNAR, M., JUAN, M., ALMELA, V. 1995b. Effect of 3,5,6 trichloro 2 pyridyloxyacetic acid on clementine early fruitlet development and on fruit size at maturity. J. Hort. Sci. 70:855 962.

AGUSTÍ, M., ZARAGOZA, S., IGLESIAS, D.J., ALMELA, V. PRIMO-MILLO, E., TALÓN, M. 2002. The synthetic auxin 3,5,6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. Plant Growth Regul., 36: 141-147

ALMELA, V., AGUSTÍ, M. AZNAR, M. 1990. El "splitting" o rajado del fruto de la mandarina 'Nova'. Su control. Act. Hort., 6: 142-147.

ALMELA, V., AGUSTÍ, M., PONS, J. 1992. Rind spots in Fortune mandarin. Origin and control. Physiol. Plant., 85:, A60 (Abstr.).

ALMELA. V., ZARAGOZA, S., PRIMO MILLO, E., AGUSTÍ, M. 1994. Hormonal control of splitting of 'Nova' mandarin fruit. J. Hort. Sci., 69: 969-973.

ALMELA, V., JUAN, M., LAPICA, P. SALVIA, J., AGUSTÍ, M. 1997. Control de la abscisión del fruto maduro en los cítricos. C. V. Agraria, 10:15-22.

ALMELA, V., SEGURA, V., GARIGLIO, N., GONZÁLEZ-PRIMO, D. JUAN, M., AGUSTÍ, M. 2000. El colapso de la corteza de las naranjas Navel. Phytoma, 119: 43-52.

AZNAR, M., ALMELA, V., JUAN, M., EL OTMANI, M., AGUSTÍ, M. 1995. Effect of the synthetic auxin phenothiol on fruit development of 'Fortune' mandarin. J. Hort. Sci. 70:617 621.

BAR-AKIVA, A. 1975. Effect of potassium nutrition on fruit splitting in 'Valencia' orange. J. Hort. Sci., 50: 85-89.

CASAS, A., MALLENT, D. 1983. Aclareo químico y modificación de la maduración de mandarinas Clausellinas con Figaron (éster etílico del ácido 5-cloro-1H-3 indazolacético). Rev. Agroquim. Tecnol. Aliment., 23: 360-368.

CLELAND, R. E. 1987. Auxin and cell elongation. P 132-148. In: P. J. Davies (ed.). Plant Hormones and their role in plant growth and development. Martinus Nijhoff, Boston.

COGGINS, C. W. JR., EAKS, I.L. 1964. Rind staining and other rind disorders of navel orange reduced by gibberellin. Calif. Citrograph, 50: 2-47.

COGGINS, C.W. JR. , HIELD, H.Z. 1968. Plant growth regulators. P. 86-126. In: W. Reuther, L.D. Batchelor and H.B. Webber (eds.). The Citrus Industry, vol. II, Univ. Calif., Div. Agr. Sci., California, USA.

COHEN, A. 1984. Citrus fruit enlargement by means of summer girdling. J. Hortic. Sci., 59: 119-125.

COHEN, A., LOMAS, J., RASSIS, A. 1972. Climatic effects on fruit shape and peel thickness in 'Marsh seedless' grapefruit. J. Amer. Soc. Hort. Sci., 97: 768-771.

COHEN, E., SHAPIRO, B., SHALOM, Y., KLEIN, J.D. 1994. Water loss: A non-destructive indicator of enhanced cell membrane permeability of chilling-injured citrus fruit. J. Amer. Soc. Hort. Sci., 119: 983-986.

COHEN, E., SHALOM, Y., ROSENBERGER, I. 1990. Post-harvest behaviour of 'Ortanique' ('Topaz') tangor citrus fruit during long-term storage at various temperatures. Scientia Horticulturae, 44: 235-240.

DAVIES, F.S., ALBRIGO, L.G. 1994. *Citrus*. CAB International, Wallingford, Oxon, UK

DE CICCO, V., INTRIGLIOLO, F., HIPOLITO, A., VANADIA, S., GIUFFRIDA, A. 1998. Factors in 'Navelina' orange splitting. Proc. Int. Soc. Citriculture, 1: 535-540.

DOESBURG, J. J. 1975. Relation between the solubilization of pectin and the fate of organic acids during maturation of apples. J. Sci. Food Agric., 8:206-216.

EL OTMANI, M., AGUSTÍ, M., AZNAR, M., ALMELA, V. 1993. Improving the size of 'Fortune' mandarin fruits by the auxin 2,4 DP. Scientia Hort. 55:283 290.

EL-OTMANI, M., COGGINS, C. W. JR. 1985. Fruit age and growth regulator effects on the quantity and structure of the epicuticular wax of ' Washington' navel orange fruit. J. Amer. Soc. Hort. Sci., 110: 371-378.

EL-OTMANI, M., COGGINS, C.W. JR., AGUSTÍ, M., LOVATT, C.L. 2000. Plant growth regulators in citriculture: World current uses. Crit. Rev. Plant Sci., 19: 395-447.

EL-OTMANI, M., LOVATT, C. L., COGGINS, C.W. JR., AGUSTÍ, M. 1995. Plant growth regulators in citriculture: Factors regulating endogenous levels in citrus tissues. Crit. Rev. Plant Sci., 14: 367-412.

ERIKSON, L. C. 1957. Compositional differences between normal and split 'Washington navel' oranges. J. Amer. Soc. Hort. Sci., 70: 257-260.

ERNER, L., MONSELISE, S.P., GOREN, R. 1975. Rough fruit condition of the Shamouti orange. Occurrence and pattern of development. Physiol. Vég., 13: 435-443.

GALLASCH, P.T. 1988. Chemical thinning of heavy crops of mandarins to increase fruit size. Proc. 6th Int. Citrus Cong. 1:395 405.

GARCÍA-LUIS, A., AGUSTÍ, M., ALMELA, V., ROMERO, E., GUARDIOLA, J.L. 1985. Effect of gibberellic acid on ripening and peel puffing in 'Satsuma' mandarin. Scientia Hort., 27: 75-86.

GOLDSCHMIDT, E.E., KOCH, K.E. 1996. Citrus. In: E. Zamski and A.A. Schaffer (eds.), *Photoassimilate distribution in plants and crops. Source-sink relationships*. Marcel Dekker, Inc. New York Basel, Hong Kong, pp 797-823.

GOLDSCHMIDT, E. E., MONSELISE, S. P. 1977. Physiological assumptions toward the development of a citrus fruiting model. Proc. Int. Soc. Citriculture, 2: 668-672.

GUARDIOLA, J. L., AGUSTÍ, M., BARBERÁ, J., SANZ, A. 1981. Influencia del ácido giberélico en la maduración y senescencia del fruto en la mandarina Clementina (Citrus reticulata Blanco). Rev. Agroquim. Tecnol. Aliment., 21: 225-239.

GUARDIOLA, J.L., LÁZARO, E. 1987. The effect of synthetic auxins on fruit growth and anatomical development in Satsuma mandarin. Scientia. Hort. 31:119 130.

GUARDIOLA, J. L., GARCÍA-LUIS, A. 2000. Increasing fruti size in citrus. Thinning and stimulation of fruit growth. Plant Growth Regul., 31: 121-132.

HOLTZHAUSEN, L. C. 1981. Creasing: Formulating a hypothesis. Proc. Int. Soc. Citriculture, 1: 201-204.

IWAHORI, S. 1978. Use of growth regulators in the control of cropping of mandarin varieties. Proc. Int. Soc. Citriculture: 263-270.

JACKSON, P.R., AGUSTÍ, M., ALMELA, V., JUAN, M. 1992. Tratamientos para mejorar la conservación en el árbol del fruto de la mandarina Fortune. Levante Agrícola, 317/318: 16-22.

JIMÉNEZ-CUESTA, M., CUQUERELLA, J., MARTINEZ-JÁVEGA, J. M. 1981. Determination of a color index for citrus degreening. Proc. Int. Soc. Citriculture, 2: 750-752.

JONES, W. W., EMBLETON, T.W., GARBER, M.J., CREE, C.B. 1967 Creasing of orange fruit. Hilgardia, 38:231-244.

KAMURO, Y., HIRAI, K. 1981. Physiological activity of ethylchlozate. Fruit thinning and maturity accelerating effects for citrus. Proc. Int. Soc. Citriculture, 1: 260-263.

KAUFMAN, M. R. 1970. Extensibility of pericarp tissue in growing citrus fruits. Physiol. Plant., 46: 778-781.

KAWASE, K., SUZUKI, K., HIROSE, K. 1981. Use of growth regulators to control rind puffing in Satsuma mandarin fruit. Proc. Int. Soc. Citriculture, 237-239.

KLOTZ, L. J., COGGINS, C.W. JR., DE WOLFE, T.A. 1966. Rind breakdown of navel oranges. Calif. Citrogr., 51: 174-196.

KURAOKA, T. 1962. Histological studies on the fruit development of the Satsuma orange with special reference to peel-puffing. Memoirs of Ehime Unviersity, Sect. VI, 8: 106-154.

KURAOKA, T., IWASAKI, K., KADOYA, K. 1966. Studies on peel puffing and levels of GA like substances and ABA in the peel of Satsuma mandarin (*Citrus unshiu* Marc.). V. Effect of GA_3 application. Proc. Jap. Soc. Horticultural Sci., Spring Meeting, 43-44.

LEWIS, L. N., COGGINS, C. W. JR., LABANAUSKAS, C.K., DUGGER, W.M. 1967. Biochemical changes associated with natural and gibberellin A3 delayed senescence of the navel orange rind. Plant Cell Physiol., 8: 151-160.

LIEGEOIS, S., PONS, J., JUAN, M., ALMELA, V., AGUSTÍ, M. 1995. Effets de différents traitements sur l'evolution de la maturation de la mandarine Marisol (*Citrus reticulata* Blanco). Fruits, 50: 117-124.

LIMA, J.E.O., DAVIES, F.S., KREZDORN, A.H. 1980. factors associated with excessive fruit drop of Navel oranges. J. Amer. Soc. Hortic. Sci., 105: 902-906.

MONSELISE, S. P., WEISER, M., SHAFIR, N., GOREN, R., GOLDSCHMIDT, E.E. 1976. Creasing of orange peel. Physiology and control. J. Hort. Sci. 51: 341-351.

MONSELISE, S. P., COSTO, J. 1985. Decreasing splitting incidence in 'Murcott' by 2,4 D and calcium nitrate. Alon Hanotea, 39: 731-733.

ORTOLA, A. G., MONERRI, C., GUARDIOLA, J.L. 1991. The use of naphtalene acetic acid as a fruti growth enhancer in Satsuma mandarin: a comparison with the fruti thinning effect. Scientia Hort., 47: 15-25.

PANTASTICO, E. B., SOULE, J., GRIERSON, W. 1968. Chilling injury in tropical and subtropical fruits: II. Limes and grapefruit. Trop. Reg. Amer. Soc. Horticultural Sci., 12 : 171-183.

PONS, J., ALMELA, V., AGUSTÍ, M. 1988. Efecto de las aplicaciones de Figaron (etilclozate) en la maduración de las mandarinas 'Oroval' (Citrus reticulata Blanco) y Satsuma (*Citrus unshiu* Marc.). Actas III Congreso SECH: 11-16.

PONS, J., ALMELA, V., JUAN, M., AGUSTÍ, M. 1992. Use of Ethephon to promote colour development in early ripening Clementine cultivars. Proc. Int. Soc. Citriculture, 1: 459-462.

RUIZ, L. L., PRIMO MILLO, E. 1989. El rajado, agrietado o "splitting" de los frutos cítricos. Levante Agrícola, 291: 98-102.

SAWAMURA, M., MANABET, T., OONISHI, S., YASUOKA, K., KUSUNOSE, H. 1984. Effects of rind oils and their components on the induction of rind spot in Citrus species. J. Hortic. Sci., 59: 575-579.

STEWART, W. S., KLOTZ, L. J. 1947. Some effects of 2,4-dichlorophenoxyacetic acid on fruit-drop and morphology of oranges. Bot. Gaz., 109: 150-162.

STOREY, R., TREEBY, M.T. 1994. The morphology of epicuticular wax and albedo cells of orange fruit in relation to albedo breakdown. Journal of Horticultural Science, 69: 329-338.

VERCHER, R., TADEO, F.R., ZARAGOZA, S., ALMELA, V., PRIMO-MILLO, E., AGUSTÍ, M. 1994. Rind structure, epicuticular wax morphology and water permeability of 'Fortune' mandarin fruits affected by peel pitting. Ann. Bot., 74: 619-625.

WHEATON, T.A. 1981. Fruit thinning of Florida mandarins using plant growth regulators. Proc. Int. Soc. Citriculture 1:263 268.

ZACARÍAS, L., ALFÉREZ, F., GARIGLIO, N., ALMELA, V., AGUSTÍ, M. 2001. Rind breakdown in Navelate oranges. The influence of rootstock. Proc. Int. Soc. Citriculture (in press).

ZARAGOZA, S., ALONSO, E. 1975. El manchado de la corteza de los agrios. Estudio preliminar en la variedad 'Navelate'. Manchas pre-recolección. Comun. INIA, Serv. Prot. Veg., 4: 5-8, 13-14, 19-22, 31-32.

ZARAGOZA, S., TENOR, I., ALONSO, E., PRIMO MILLO, E., AGUSTÍ, M. 1992. Treatments to increase the final fruit size on *Satsuma Clausellina*. Proc. Int. Soc. Citriculture 2:725 728.

ZARAGOZA, S., ALMELA, V., TADEO, F.R., PRIMO-MILLO, E., AGUSTÍ, M. 1996. Effectiveness of calcium nitrate and GA_3 on the control of peel-pitting of 'Fortune' mandarin. J. Hort. Sci., 71: 321-326.