

Fruit set dependence on carbohydrate availability in citrus trees

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Summary We generated source–sink imbalances by defoliation and sucrose supplementation by stem injection, to investigate effects of carbohydrate availability on fruitlet growth and abscission in cv. Okitsu of Satsuma mandarins (*Citrus unshiu* (Mak.) Marc.). Partial defoliation promoted fruitlet abscission, whereas sucrose supplementation increased citrus fruit set by more than 10%. Moreover, when applied together, sucrose supplementation counteracted the effect of partial defoliation on fruit set. When sucrose was supplied continuously from flowering until harvest, it increased the concentrations of soluble and insoluble sugars in fruits. We conclude that fruit set in citrus is highly dependent on carbohydrate availability.

Keywords: carbon availability, defoliation, fruit abscission, source–sink imbalances, sucrose supplementation.

Introduction

Citrus trees flower profusely. After flowering, abscission of many developing ovaries is followed by a wave of fruitlet abscission (Goldschmidt 1999). Consequently, the number of flowers produced by *Citrus* species is much higher than the number of fruits at harvest.

The first abscission wave of developing ovaries is known to have a strong hormonal component, with gibberellin playing a pivotal role in the process (Talon et al. 1990, 1992). In fruit trees (Gillaspy et al. 1993), including citrus (Ruiz et al. 2001), fruitlet abscission during the June drop is particularly dependent on metabolic factors. There is circumstantial evidence that developing fruitlets compete for photoassimilates (Goldschmidt and Koch 1996), and it has been suggested that fruits that do not reach a certain threshold carbohydrate concentration have a high probability of abscission (Mehouachi et al. 1995, Talon et al. 1997). Recently, it has also been demonstrated that carbon shortage induces fruit abscission through a characteristic hormonal sequence of which the final product is ethylene (Gómez-Cadenas et al. 2000).

Indirect evidence that carbohydrates have a role in the abscission process comes from several studies. Experiments involving translocation of ¹⁴C-metabolites (Powell and Krezdorn 1977) and CO₂-enriched atmospheres (Downton et al.

1987, Idso et al. 1991) showed positive effects of carbon availability on both vegetative and reproductive development. Furthermore, leaf photosynthesis, and hence photosynthate production, appears to be crucial in determining fruit set (Buwalda and Smith 1990, Mehouachi et al. 2000). A decrease in leaf number as a result of defoliation (Mehouachi et al. 1995) or a reduction in photosynthetic activity in response to shading (Jackson and Palmer 1977) has been correlated with increased fruitlet abscission rates and, therefore, decreased fruit set. On the other hand, girdling treatments decrease fruitlet abscission, probably by blocking photoassimilate transport from leaves to roots, thus increasing carbohydrate availability for the growing fruitlets (Yamanishi 1995). Similarly, fruit thinning may decrease fruitlet abscission by increasing the availability of carbon among the remaining fruit (see Goldschmidt and Koch 1996). After the June drop, few citrus fruits abscise until maturity.

These observations strongly suggest that there is a relationship between the nutritional status of the tree and fruit set, although direct evidence of the role of carbohydrates in fruitlet abscission is lacking. Recently, we reported that sucrose supplementation produces various physiological effects in citrus fruits (Abdin et al. 1998, Iglesias et al. 2001). In the present work, we investigated the influence of sucrose supplementation through successive stem injections on citrus fruit set.

Materials and methods

Plant material

Seven-year-old trees (100 to 125 cm tall) of Satsuma mandarin (*Citrus unshiu* (Mak.) Marc.) cv. Okitsu were used. This cultivar belongs to the Satsuma Wase group, shows moderate dwarfism and carries developing fruitlets in leafy and leafless inflorescences, following the usual pattern observed in Satsuma. Under normal conditions, anthesis in this cultivar takes place in early April.

Defoliation treatments

Defoliation consisted of removing 66% of the leaves, and was performed either 10 or 35 days after anthesis (DAA). Newly

produced sprouts were removed periodically every 2 weeks to reduce competition between fruit development and leaf regrowth.

In vivo sucrose supplementation

The method for *in vivo* sucrose supplementation has been described in detail by Iglesias et al. (2001). Briefly, sucrose was injected into stems with a low-pressure injection system consisting of a plastic injector and a 250-ml rubber reservoir providing pressures of 60–80 kPa. In general, successive injections of 292 mM sucrose solution into plants were performed until harvest. Sucrose injections started at either 30 days before anthesis (–30 DAA) or 35 DAA. All sucrose experiments began with more than 10 trees per treatment, although data are presented only for the five plants that absorbed the highest quantities of sucrose.

Carbohydrate analyses

Fruits were randomly sampled for carbohydrate analysis from 40 to 120 DAA. The material was lyophilized, ground and stored at –20 °C until analyzed for carbohydrates as previously described (Mehouachi et al. 1995). Briefly, samples were extracted with 80% ethanol and purified sequentially by cation and anion exchange columns, and through C₁₈-cartridges. Sucrose, glucose and fructose were analyzed with a Waters HPLC system equipped with a high performance carbohydrate column (4.6 × 250 mm, Waters Cromatografía, Barcelona, Spain) and a 2410 differential refractometer. Starch was determined in the remaining pellets after extraction of soluble sugars. Starch was gelatinized by autoclaving the samples, sodium-acetate buffer and amyloglucosidase were added to the extracts, and enzymatic digestions were performed for 2 h at 55 °C. After filtration, released glucose was determined by HPLC as described above.

Reproducibility of the determinations and statistical analyses

Experiments involving sucrose supplementation to non-defoliated trees were performed in four consecutive seasons with similar results, although only representative sets of data from a particular year are presented. Partial defoliation treatments were performed at 10 and 35 DAA, and repeated twice in consecutive years. In general, experiments were replicated in four blocks with a minimum of five trees per block. Fruitlet drop was periodically recorded on populations of 100 tagged fruitlets, usually at 7-day intervals. Abscission rates were calculated as the proportion of dropped fruitlets each week relative to the number present at the beginning of that week. Total yield was determined by counting and weighing all remaining fruits at harvest (200–220 DAA).

Changes in carbohydrate concentrations were determined in samples from 40 DAA until the ripening period (120 DAA). The effectiveness of the sugar extractions was ascertained with fucose as an internal standard. Sequential extractions were performed to quantify sugar concentrations. With this method, the amounts of internal standard added to the extractions were successively adjusted in consecutive extractions until similar

amounts of standards and endogenous compounds were found in the samples (Mehouachi et al. 2000). At least three independent extractions per sample were performed, and the data presented correspond to the mean of three samples.

Parameters were statistically tested by analyses of variance, and comparisons of means were determined with a Student's *t*-test ($P \leq 0.05$). Statistical analyses were performed with Statgraphics Plus for Windows, Version 2.1 (Statistical Graphics, Englewood Cliffs, NJ).

Results

Effects of sucrose on fruitlet abscission and fruit yield

In general, fruitlet abscission in Okitsu trees increased continuously from anthesis to about 80 DAA. Although continuous stem injections of 292 mM sucrose started at –30 DAA, no effects were detected until 60–65 DAA (end of June; see Figure 1). Sucrose-treated trees exhibited decreased fruitlet abscission, and maximum differences between sucrose-treated and untreated trees (13.2 versus 25.7%, respectively) were reached at 90–95 DAA. By this time, each treated tree had absorbed about 30 g of sucrose. The experiment was repeated in four consecutive seasons, with similar results. Sucrose supplementation reduced fruitlet abscission by 10–15%.

Defoliations (66%) performed at 35 DAA increased fruitlet abscission from 25.7 to 42.8% (Figure 2). Sucrose treatment decreased fruitlet abscission in the partially defoliated trees by about 15% relative to non-supplemented, partially defoliated trees. The partially defoliated trees treated with sucrose absorbed about 30 g of sucrose by 90–95 DAA.

The effect of increased fruit set in response to sucrose supplementation was also evident during assessment of yield. The final number of fruits per tree at harvest was increased by su-

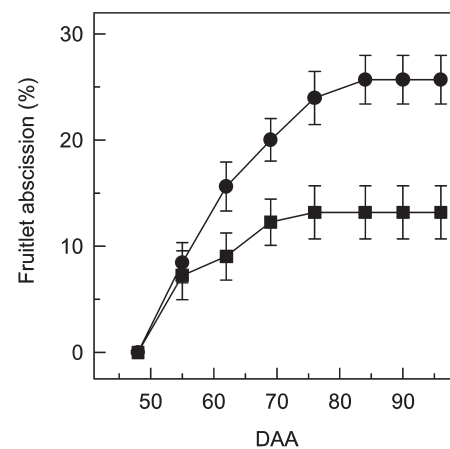


Figure 1. Effect of sucrose on fruitlet abscission in cv. Okitsu trees. The sucrose treatment started at 30 days before anthesis (–30 DAA) and consisted of continuous injections of 292 mM sucrose. After the June drop, each tree had absorbed a minimum of 30 g of sucrose. The experiment was repeated in four consecutive seasons with similar results. Data are means ($n \geq 5$ trees with at least 100 tagged fruitlets per tree) \pm SE. Symbols: ● = control; and ■ = sucrose.

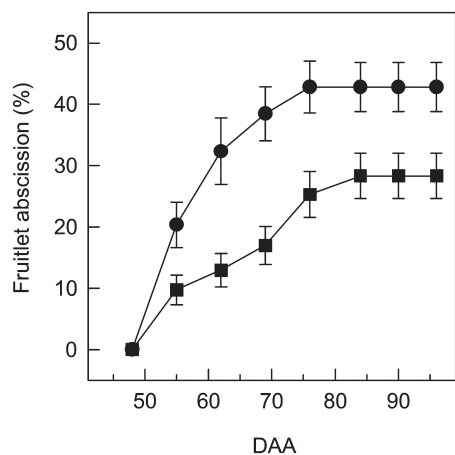


Figure 2. Effect of sucrose on fruitlet abscission in defoliated (66%) cv. Okitsu trees. Defoliation was performed at 35 days after anthesis (DAA). The sucrose treatment started at 30 days before anthesis (–30 DAA) and consisted of continuous injections of 292 mM sucrose. After the June drop, each tree had absorbed a minimum of 30 g of sucrose. The experiment was repeated in four consecutive seasons with similar results. Data are means ($n \geq 5$ trees with at least 100 tagged fruitlets per tree) \pm SE. Symbols: ● = control; and ■ = sucrose.

crose in both partially defoliated and non-defoliated trees. Sucrose supplementation in non-defoliated trees increased fruit yield about 13% (Figure 3). The pattern was similar in other experiments, with increases in yield ranging between 10 and 15% (data not shown). To study the effect of sucrose supplementation on partially defoliated trees, 66% of leaves were removed at 35 DAA, and the sucrose treatment was initiated at either –30 or 35 DAA. Compared with non-supplemented partially defoliated trees, a positive effect on fruit number was observed when partially defoliated trees were injected with su-

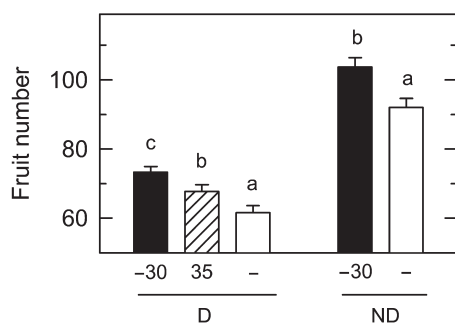


Figure 3. Effect of the timing of sucrose treatment on fruit number in 66% defoliated (D) and non-defoliated (ND) trees. The defoliation treatment was performed 35 days after anthesis (DAA) and data were obtained at harvest (200–220 DAA). By this time, sucrose-treated trees had absorbed a minimum of 30 g of sucrose. Sucrose treatment started at either 30 days before anthesis (–30 DAA; solid bars) or 35 DAA (hatched bars); open bars are the control. Newly produced sprouts were regularly removed. Data are means ($n \geq 5$ trees) \pm SE. In each experiment, different letters indicate statistically significant differences ($P \leq 0.05$).

crose from –30 DAA or 35 DAA (Figure 3).

Sucrose supplementation resulted in increases in fruit yield in trees partially defoliated at 10 or 35 DAA (Figure 4). In non-defoliated trees, sucrose supplementation increased fruit number by 12%, whereas in partially defoliated trees, the sucrose-induced increase was slightly higher (16 and 23%). Similar tendencies were observed in complementary experiments performed the following season.

Effects of sucrose supplementation on fruit set and yield

To determine the effect of the amount of sucrose supplementation, trees were treated regularly with different amounts of sucrose from –30 DAA until the sucrose absorbed per tree was 3, 30 or 50 g (Figure 5). Sucrose supplementation decreased fruitlet abscission by 11–19% at the end of the June drop. Final fruit number and total fruit yields corresponding to differential sucrose supplementation treatments are shown in Table 1. As expected, plants treated with sucrose had a higher fruit load than untreated trees (between 17 and 27%), although no significant differences were found between treatments.

Effect of sucrose supplementation on fruit carbohydrate concentrations

Soluble sugar concentrations of fruits increased during the growing period in both non-defoliated and partially defoliated trees (Figure 6). In general, partial defoliation did not result in statistically significant decreases in concentrations of soluble sugars in fruits. Sucrose supplementation increased the hexose concentration of fruits of both non-defoliated and partially defoliated trees, but significantly increased the sucrose concentration of fruits only in samples taken at 40 and 120 DAA from non-defoliated trees.

Starch concentrations of fruits showed no marked differences during the study period (Figure 6). The minimum starch concentration, expressed as mg of released glucose per g dry weight, was reached at 40 DAA in both non-defoliated and defoliated trees. Thereafter, starch concentration in fruits was

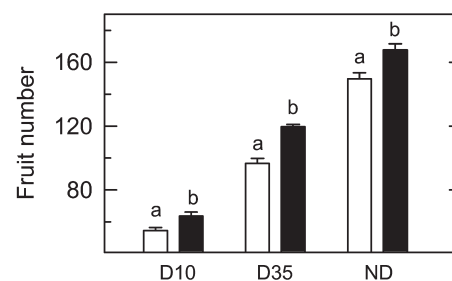


Figure 4. Effect of sucrose on fruit number in non-defoliated (ND) and 66% defoliated cv. Okitsu trees. Defoliations were performed 10 or 35 days after anthesis (D10 and D35, respectively), simultaneously with sucrose supplementation (□ = control; ■ = sucrose). Values are means ($n \geq 5$ trees) \pm SE of data obtained at harvest (200–220 days after anthesis) from the experiments in Figures 1 and 2. Newly produced sprouts were regularly removed. In each experiment, different letters indicate statistically significant differences ($P \leq 0.05$).

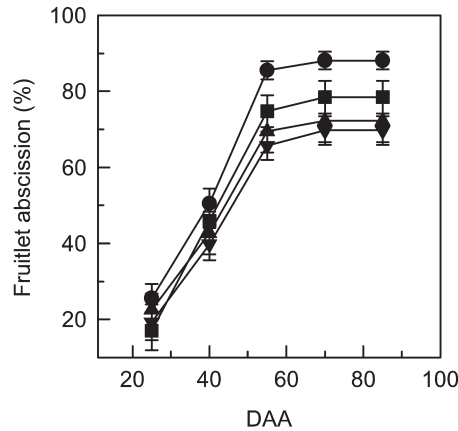


Figure 5. Effect of amounts of sucrose on fruitlet abscission in cv. Okitsu trees. After the June drop, the amounts of sucrose absorbed per tree were approximately 3 (■), 30 (▲) and 50 g (▼) (● = control). The sucrose treatment started at 30 days before anthesis (–30 DAA) and consisted of continuous injections of different sucrose concentrations. Data are means ($n \geq 5$ trees with at least 100 tagged fruitlets per tree) \pm SE.

similar in non-supplemented trees and non-defoliated trees or slightly decreased in defoliated trees. In general, fruits from sucrose-supplemented plants had higher starch concentrations than fruits from non-supplemented plants.

Discussion

Increased carbohydrate availability to growing citrus fruitlets was associated with a decreased probability of abscission during fruit set, resulting in a greater number of fruits at the end of the growing period. Citrus fruit abscission is dependent on environmental, nutritional and hormonal factors (Gillaspy et al. 1993). Nutritional factors are limiting factors, whereas hormonal compounds are effectors of the regulation of the abscission process (Gómez-Cadenas et al. 2000). Several studies on source–sink imbalances in citrus species support the hypothesis that competition for photoassimilates among developing fruitlets regulates fruit abscission. Effects of defoliation, girdling, defruiting or fruit thinning have provided indirect evidence for the role of carbohydrates in various physiological processes related to fruit growth (Goldschmidt and Koch 1996). However, each of these treatments, in addition to altering the carbohydrate pool, also appears to modify a different physiological process (see Goldschmidt 1999). The *in vivo* sucrose supplementation approach used in this study provides a means to test the direct role of carbohydrates during fruit development (Abdin et al. 1998, Iglesias et al. 2001). We combined defoliation with sucrose supply to decrease and increase, respectively, the carbohydrate supply for growing fruits. We found a clear relationship between sucrose supplementation and fruitlet abscission (Figures 1 and 2). Sucrose supplementation decreased fruitlet abscission rate in partially defoliated and non-defoliated trees, and this decrease was coincident with an increase in sugar concentrations (Figure 6).

Table 1. Number and total mass of fruits per tree at harvest in Satsuma mandarins cv. Okitsu supplemented with different amounts of sucrose. The sucrose treatment started at 30 days before anthesis and consisted of continuous injections performed until harvest. Data are means ($n \geq 5$ trees), and different letters within a row indicate statistically significant differences ($P \leq 0.05$).

	Sucrose absorbed (g)			
	0 (control)	3	30	50
Fruit number	93.8 a	110.3 b	115.7 b	119.5 b
Total mass (kg)	10.9 a	13.0 b	14.6 b	14.3 b

We note that the supply of sucrose did not compensate fully for the effect of partial defoliation (Figures 3 and 4), suggesting that partial defoliation may affect other metabolic or physiological factors, such as hormones, water relations or gas exchange.

The increase in fruit yield in response to sucrose supplementation (Figures 3 and 4, Table 1) was apparently a result of decreased fruitlet abscission before and during the June drop. These findings suggest that carbohydrates were near threshold values in fruitlets during the earliest stages of growth and that fruit carbohydrate concentration determines the probability of abscission (Talon et al. 1997). Based on ^{14}C -translocation experiments, Powell and Krezdorn (1977) concluded that the carbohydrate status of fruitlets may be a major factor determining abscission, not only in citrus (Mehouachi et al. 1995) but also in other tree species (e.g., Berüter and Droz 1991, Nzima et al. 1999). Our data indicated that sucrose supplementation during the early stages of fruit development resulted in higher yields than sucrose supplementation during later stages of fruit development (Figure 3); however, at any particular time, the process appeared to approach saturation at sucrose doses above 3 g per tree (Figure 5, Table 1).

The effect of sucrose supplementation on fruiting was accompanied by increases in fruit carbohydrate concentrations. During the initial stages of fruit development, increases in soluble sugar concentrations were small or nonsignificant, but during later stages of fruit development, the increases were significant; this is consistent with the fruitlets acting first as utilization sinks, and thereafter as storage sinks (Mehouachi et al. 1995). Moreover, it has been shown that exogenous sucrose increases concentrations of sucrose in the peel of ripening fruits (Iglesias et al. 2001), and of soluble and insoluble sugars in leaves (Iglesias et al. 2003). In our experiments, sucrose supplementation also increased starch concentrations in fruits.

The specific role of carbohydrates in fruitlet abscission is not completely understood. It has been suggested that carbohydrates and hormones participate in a complex signal transduction system (Zhou et al. 1998, Roitsch 1999). We have recently shown that carbohydrate shortage induces increases in abscisic acid and 1-amino-cyclopropane-1-carboxylic acid concentrations concomitant with fruitlet abscission and that, in citrus fruits, ethylene is the final effector of abscission (Gómez-Cadenas et al. 2000). Modulation of sugar effects by

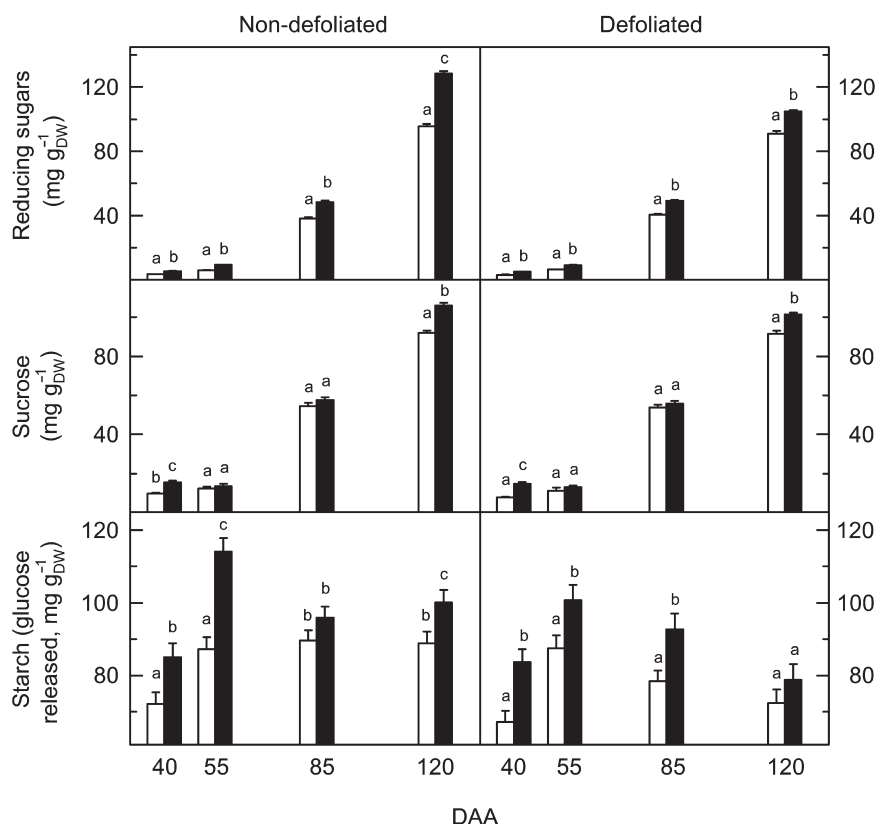


Figure 6. Reducing sugars (glucose + fructose), sucrose and starch in fruitlets from non-defoliated and 66% defoliated trees supplemented with sucrose (solid bars) or non-supplemented (open bars). Samples were obtained from the experiments in Figures 1 and 2. Data are means ($n \geq 3$ independent extractions) \pm SE. Different letters in samples harvested on the same date in both non-defoliated or defoliated trees indicate statistically significant differences ($P \leq 0.05$).

hormones, and vice versa, has also been reported for several plant hormones including gibberellins and ethylene (Zhou et al. 1998, Roitsch 1999). Thus, carbohydrates may act not only as essential nutrient factors, but also as signals triggering specific responses.

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References

- Abdin, O.A., X. Zhou, B.E. Coulman, D. Cloutier, M.A. Faris and D.L. Smith. 1998. Effect of sucrose supplementation by stem injection on the development of soybean plants. *J. Exp. Bot.* 49: 2013–2018.
- Berüter, J. and P. Droz. 1991. Studies on locating the signal for fruit abscission in the apple tree. *Sci. Hortic.* 46:201–214.
- Buwalda, J.G. and G.S. Smith. 1990. Effects of partial defoliation at various stages of the growing season on fruit yields, root growth and return bloom of kiwifruit vines. *Sci. Hortic.* 42:29–44.
- Downton, W.J.S., W.J.R. Grant and B.R. Loveys. 1987. Carbon dioxide enrichment increases yield of Valencia orange. *Aust. J. Plant Physiol.* 14:493–501.
- Gillaspy, G., H. Ben-David and W. Gruissem. 1993. Fruits: a developmental perspective. *Plant Cell* 5:1439–1451.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. *Hortic. Sci.* 34: 1020–1024.
- Goldschmidt, E.E. and K.E. Koch. 1996. Citrus. *In Photoassimilate Distribution in Plants and Crops: Source–Sink Relations*. Eds. E. Zaminski and A.A. Schaffer. Marcel Dekker, New York, pp 797–823.
- Gómez-Cadenas, A., J. Mehouchi, F.R. Tadeo, E. Primo-Millo and M. Talon. 2000. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. *Planta* 210:636–643.
- Idso, S.B., B.A. Kimball and S.G. Allen. 1991. Net photosynthesis of sour orange trees maintained in atmospheres of ambient and elevated CO₂ concentration. *Agric. For. Meteorol.* 54:95–101.
- Iglesias, D.J., F.R. Tadeo, F. Legaz, E. Primo-Millo and M. Talon. 2001. *In vivo* sucrose stimulation of colour change in citrus fruit epicarp: interactions between nutritional and hormonal signals. *Physiol. Plant.* 112:244–251.
- Iglesias, D.J., I. Lliso, F.R. Tadeo and M. Talon. 2003. Regulation of photosynthesis through source:sink imbalance in citrus is mediated by carbohydrate content in leaves. *Physiol. Plant.* In press.
- Jackson, J.E. and J.W. Palmer. 1977. Effects of shade on the growth and cropping of apple trees. II. Effects on components of yield. *J. Hortic. Sci.* 52:253–266.
- Mehouchi, J., D. Serna, S. Zaragoza, M. Agustí, M. Talon and E. Primo-Millo. 1995. Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107:189–197.
- Mehouchi, J., D.J. Iglesias, F.R. Tadeo, M. Agustí, E. Primo-Millo and M. Talon. 2000. The role of leaves in citrus fruitlet abscission: effects on endogenous gibberellin levels and carbohydrate content. *J. Hortic. Sci. Biotechnol.* 75:79–85.

- Nzima, M.D.S., G.C. Martin and C. Nishijima. 1999. Effect of fall defoliation and spring shading on shoot carbohydrate and growth parameters among individual branches of alternate bearing 'Kerman' Pistachio tree. *J. Am. Soc. Hortic. Sci.* 124:52–60.
- Powell, A.A. and A.H. Krezdorn. 1977. Influence of fruit-setting treatments on translocation of ^{14}C -metabolites in citrus during flowering and fruiting. *J. Am. Soc. Hortic. Sci.* 102:709–714.
- Roitsch, T. 1999. Source–sink regulation by sugar and stress. *Curr. Opin. Plant Biol.* 2:198–206.
- Ruiz, R., A. García-Luis, C. Monerri and J.L. Guardiola. 2001. Carbohydrate availability in relation to fruitlet abscission in Citrus. *Ann. Bot.* 87:805–812.
- Talon, M., L. Zacarías and E. Primo-Millo. 1990. Hormonal changes associated with fruit set and development in mandarins differing in their parthenocarpic ability. *Physiol. Plant.* 79:400–406.
- Talon, M., L. Zacarías and E. Primo-Millo. 1992. Gibberellins and parthenocarpic ability in developing ovaries of seedless mandarins. *Plant Physiol.* 99:1575–1581.
- Talon, M., F.R. Tadeo, W. Ben-Cheikh, A. Gómez-Cadenas, J. Mehouchi, J. Pérez-Botella and E. Primo-Millo. 1997. Hormonal regulation of fruit set and abscission in citrus: classical concepts and new evidence. *Acta Hortic.* 463:209–217.
- Yamanishi, O.K. 1995. Trunk strangulation and winter heating effects on fruit size, internal quality and maturation of 'Tosa Buntan' pummelo grown in a plastic house. *J. Hortic. Sci.* 70:65–74.
- Zhou, L., J.C. Jan, T.L. Jones and J. Sheen. 1998. Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant. *Proc. Natl. Acad. Sci.* 95:10,294–10,299.