Girdling increases carbohydrate availability and fruit-set in citrus cultivars irrespective of parthenocarpic ability

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SUMMARY
The effects of girdling performed at various dates were evaluated during two consecutive years in high- and low-bearing commercial orchards of ‘Fortune’ mandarin and ‘Clausellina’ Satsuma mandarin. The time-dependent response was evaluated through fruitlet abscission, final fruit-set and yield as related to carbohydrate contents in developing fruitlets. A few days after treatment, girdling increased the soluble sugars content (SSC) in fruitlets, reduced the daily fruit drop, and thereby diminished abscission. Application of girdling to low-bearing ‘Fortune’ mandarin orchards was most effective 15 d before anthesis (DBA) and 35 d after anthesis (DAA). It increased yield by 125%. In high-bearing orchards, the best results were achieved by girdling 35 DAA, which increased yield by 28%. In both orchards, the effect of girdling declined gradually from 35 DAA to the end of fruit drop. SSC values were found to be positively correlated with fruit-set at 25 DAA, but this tendency was reversed by 50 DAA. This study showed that the effects of girdling are time-dependent, and that yield can be improved in cultivars of different parthenocarpic ability.

It has been suggested that during Citrus development, flowers and fruitlets compete strongly with one another and with vegetative growth, for metabolites. It has been shown that, under high flowering intensities, trees cannot sustain all developing structures, which triggers an abscission process that, in turn, regulates fruit load (Agustí et al., 1982a; Rivas et al., 2004). This “competition hypothesis” has been reviewed by many researchers (Moss et al., 1972; Goldschmidt and Monselise, 1977; Powell and Krezdon, 1977; Talón et al., 2000). The hypothesis supports the idea that carbohydrate status plays a pivotal role in determining the fruit-setting capability of a tree by modulating its abscisic acid, 1-amino-cyclopropane-1-carboxylate and ethylene levels, thereby adjusting the fruit load to match the carbohydrate supply (Talón et al., 2000).

During the period of physiological fruit drop, the number of fruitlets remaining correlates negatively with fruit growth rate. This is attributed to the high demand for carbohydrate by the developing organs. Translocation of carbohydrates to the fruitlets depends on remobilisation of reserves, supply from leaves through de novo synthesis (Kozlowsky and Pallard, 1997; Goldschmidt and Koch, 1996; Lakso et al., 1999; Iglesias et al., 2002), transport, sink strength, and cleavage into hexoses through enzymatic activity (Quick and Schaffer, 1996).

From an agronomic point of view, cultural practices such as girdling can improve carbohydrate availability and thus increase fruit-set and yield (Monselise et al., 1972; Cohen, 1981; Garcia-Luís et al., 1988; Barry and Bower, 1997; Goldschmidt, 1999; Goren et al., 2003; Rivas et al., 2004). Girdling has also been shown to alter the partitioning of photosynthates, mineral nutrients and plant growth regulators in the tree (Furr and Armstrong, 1956; Fishler et al., 1983; Mataa et al., 1998; Wallerstain et al., 1973; 1974; 1978ab).

Most girdling experiments have been applied to low-bearing cultivars, and have not included comparisons with high-bearing cultivars. Moreover, although girdling has proved to be effective at increasing yield (Gravina et al., 1998; Rivas et al., 2004), there is a lack of information about the most appropriate time for girdling, and the reasons for its effects.

The aim of the present study was to examine the effects of time of girdling on fruit-set, abscission pattern and yield. The relationships between starch, sucrose and reducing sugars contents in developing fruitlets of ‘Fortune’ and ‘Satsuma’, two mandarin cultivars that differ in their productivity, provided information about the effects of girdling.

MATERIALS AND METHODS

Experiment 1
Plant material and treatments: Commercial orchards of ‘Fortune’ mandarin (‘Clementine’ mandarin × ‘Dancy’ tangerine) grafted onto ‘Carrizo’ citrange rootstock (Citrus sinensis [L.] Osbeck × Poncirus trifoliata Raf.)
were chosen for Experiment 1. Orchards differing in yield were located in Cheste (Valencia) and Onda (Castellón) and had average yields of 40 and 80 kg per tree, respectively. Trees aged 7 years, under drip irrigation and growing in sandy-loamy soil were used. Individual trees were selected to have similar size, vigour, crop load and flowering intensity the following season. Girdling was performed on the main scaffold branches (5–7 cm diameter) in two consecutive years. Sharp hooked-bladed scissors were used to make a cut 1 mm-wide completely encircling the branch, without reaching the xylem and not removing any bark. Different trees were used each year. Anthesis was defined as when 60% of the flowers were open. Girdling was applied 15 d before anthesis (15 DBA), at anthesis (A), 10 d after anthesis (10 DAA), 35 DAA, 60 DAA and 90 DAA. The last girdling coincided with the end of fruitlet drop. A set of ungirdled trees served as controls.

Fruitlet abscission and yield: In the first year, 30 DBA in the low-yielding orchard, four branches per tree, with about 800 nodes per branch, were tagged. Numbers of flowers and fruitlets were counted until the end of natural fruitlet drop. Evaluations were made at the same time as girdling was performed. The percentage fruit-set for each sampling date was calculated as the number of fruitlets remaining on the tree compared to the total number of flowers on the branch. Fruit number, weight and yield per tree for each treatment in both orchards were recorded at harvest.

Carbohydrate analysis: In each block, fruitlets (ca. 10) were collected 25 and 50 DAA from control trees and from 15 DBA and A girdled trees for carbohydrate analysis. Samples were frozen immediately in liquid N₂, lyophilised and stored as powders at –28°C. Samples (100 mg) of dry matter were extracted with 1.0 ml 80% (v/v) ethanol with 0.1 ml fucose (60 mg ml⁻¹) as an internal standard. The extracts were incubated at 85°C for 5 min and centrifuged at 15,000 × g for 10 min. The supernatant was removed, the pellet was re-extracted twice as above, and the combined supernatants were evaporated to 0.5 ml in vacuo at 45°C. Sequential columns of Cation Dowex 50 X (80-100-mesh) and Anion 1 X (40-mesh) (Sigma Chemicals Co. Inc., St Louis, MO, USA) were prepared for removal of sugars. Syringes (5 ml) were filled to 1 ml with the resin. The cation exchanger was washed with 2M HCl and double-distilled water to equilibrate the pH to 4.5. The anion exchanger was washed with 1M Na₂CO₃ and water until equilibrated to pH 7.5. The concentrated extract (0.5 ml) was loaded onto the cation exchanger, and an extra 0.1 ml distilled water was used to rinse the tube. The solution was eluted from the cation column into the anion column, and an extra 0.4 ml double-distilled water was used to wash the anion column. The eluate solution from the anion column, together with the extra 0.4 ml distilled water wash, was collected for further identification. The eluates were filtered through a 0.45 μm membrane (Waters-Millipore, Barcelona, Spain), then passed through a C18 Sep-Pak cartridge (Waters-Millipore). Samples were dried in vacuo at 45°C and redissolved in 100 μl double distilled water. Sugars were analysed with a Spectra HPLC System® (Spectra, San Jose, California, USA) connected to a differential refractometer (Spectra R150), vacuum pump (Spectra P2000) and ChromQuest® software system for data processing (Thermo Quest Inc., San Jose, CA, USA). Twenty μl aliquots of the filtered extracts were injected into an APS-2 Hypersil 250 × 4.6 mm column attached to a longguard pre-column (20 × 0.65 mm). A solution of acetonitrile and water [60:40 (v/v)] was used as solvent at a flow rate of 1 ml min⁻¹ for a 20 min run. Sucrose, glucose and fructose were identified according to their retention times. The recovery of sugars was evaluated by comparison with that of fucose.

Starch levels were determined in the pellets that remained after the extraction of soluble sugars. The residue was dissolved in 6.0 ml water, gelatinised by autoclaving for 2 h at 135°C, centrifuged for 10 min at 27,000 × g, and 4 ml of the supernatant removed. The sample was incubated with shaking for 2 h at 55°C with 0.2 ml fucose (60 mg ml⁻¹) as an internal standard, 0.5 ml sodium acetate pH 4.5 and 1 ml amyloglucosidase (1218 Units; from Rhizopus, Sigma Chemical Co. Inc.). Samples were centrifuged for 30 min at 27,000 × g and the supernatant was dried in vacuo and redissolved in 1 ml water. The glucose released was determined by HPLC as above.

Statistical design: Trials were carried out in a randomised complete-block design with five blocks and a single tree per treatment per block. The results were subjected to ANOVA in order to determine their statistical significance, and means were compared by means of Duncan’s New Multiple Range Test.

Experiment 2
Plant material and treatments: Ten-year-old Satsuma mandarin cv. ‘Clausellina’ (C. unshiu) grafted onto ‘Carrizo’ citrange rootstock (C. sinensis [L.] Osbeck × P. trifoliata Raf) were selected. The trees were grown in Moncada (Valencia), on sandy-loamy soil, under drip irrigation. Girdling (as described earlier) was applied to the main scaffold branches of trees at 10 and 40 DAA. A set of ungirdled trees served as controls.

Fruitlet abscission analysis: Four branches per tree, chosen at random, were tagged and the numbers of flowers were recorded at 10 d intervals until the end of abscission. Fruits on the tree were counted to monitor the abscission process.

Carbohydrate analysis: In each block, approx. 5 fruitlets from the girdling treatment 40 DAA, and from the control were collected at 20 d intervals. The samples were frozen immediately in liquid N₂, lyophilised, powdered and stored at –28°C. Carbohydrate analyses were as described earlier. Fruit number, weight and yield per tree, for each treatment were recorded at harvest.

Statistical design: This trial was conducted in a randomised complete-block design with ten blocks and a single tree per treatment per block. The results were subjected to ANOVA in order to determine their statistical significance, and means were compared by Duncan’s New Multiple Range Test.
RESULTS
Effects of girdling on fruit-set and abscission pattern

In both cultivars, girdling delayed the abscission of fruitlets during the physiological drop process, and enhanced final fruit-set (Figure 1; Figure 2). These effects were observed in all treatments.

In ‘Fortune’ mandarin (Figure 1), 10 DAA, control trees were found to have only 52% fruit-set, whereas those that had been girdled at anthesis (A) had 62% fruit-set. At 35 DAA, control trees had 8% set, whereas those that were girdled at A and 10 DAA had 14% and 12.5% fruit-set, respectively. Moreover, 60 DAA, fruit-set on control trees was only 1.5% compared with almost 6% on the 35 DAA trees. Fruit-set on trees girdled 15 DBA was always higher than on control trees and, at the end of physiological fruit drop, trees that had been girdled at 15 DBA were found to have the highest fruit-set (> 1.1%); whereas those girdled at A, 10 DAA and 35 DAA maintained between 0.5 – 0.7% fruit-set. Control trees had only 0.2% fruit-set.

In ‘Clausellina’ mandarin, the setting pattern was similar to that in ‘Fortune’ mandarin. Irrespective of the date on which it was performed, girdling delayed fruit drop (Figure 2A). Twenty DAA, trees that had been girdled 10 DAA had 8% abscission compared with almost 25% in the controls (Figure 2A), and the abscission rates of control and girdled trees at 20 DAA were 2.3 and 0.4 fruitlets d⁻¹, respectively, and showed the greatest difference (Figure 2B). Thirty DAA the total percentages of abscission in girdled and control trees were 19% and 40%, respectively. The differences in abscission decreased until 55 DAA, then remained constant until harvest (Figure 2A). The final fruitlet abscission in trees that were girdled at 10 DAA did not differ significantly from that of the controls, but the increase in daily abscissions was significantly delayed by girdling (Figure 2B). When girdling was performed 40 DAA, the differences in fruit drop started to be significant 15 d later, and additional abscission was almost prevented (Figure 2A). Similar to the case of girdling at 10 DAA, the time-course of daily fruitlet abscission was significantly altered by girdling 40 DAA (Figure 2B), but a significant increase in final fruit-set was achieved.

Effects of girdling on fruitlet carbohydrate contents and on fruit-set

In ‘Fortune’ mandarin there were significant differences in sucrose content between treatments on both sampling dates. On the first measurement date (25 DAA), girdling at 15 DBA, and at A, increased sucrose contents 1.5- and 2.4-fold, respectively, compared with
the control (Table I). This relationship was reversed at 50 DAA. No differences in sucrose content were found between sampling dates for the control, but both girdling treatment were significantly different from the control. Reducing sugars (glucose + fructose) contents were not significantly different between treatments or between dates.

Starch contents were not modified significantly by girdling, being approx. 45 mg g⁻¹ and 27 mg g⁻¹ DW for all treatments at 25 DAA and 50 DAA, respectively.

Twenty-five DAA there was a positive and significant relationship between fruit-set and sucrose concentration \((r = 0.88)\). By 50 DAA, this relationship was inverted, and a significant negative correlation was found between fruit-set and sucrose concentration \((r = -0.88)\). Fruit growth-rates on control trees remained almost constant over the period of measurement, and sucrose contents did not change significantly; whereas the fruits on girdled trees showed changed sucrose concentrations during this growth period, and their growth rate was somewhat slower than that of the controls (data not shown). Data suggest that girdling delayed abscission, but resulted in greater fruit-set (Table I; Figure 1) and a higher number of harvested fruit (Figure 3). Girdling of ‘Clausellina’ changed the sucrose levels in developing fruitlets (Figure 4). Ten d after girdling, sucrose contents were greater than those in the controls, and remained so until the end of fruit drop. The differences between treatments were observed to be greatest at 70 DAA, and slightly lower by 85 DAA.

Glucose and fructose contents also increased during the sampling period (Figure 4B, C), but remained lower than that of sucrose (Figure 4A). Differences in glucose and fructose concentrations were found to be higher on girdled trees, and were greatest at 70 DAA.

Starch contents in fruitlets on girdled and control trees remained nearly constant, varying between 18 – 22 mg g⁻¹ DW.

**Effects of girdling on yield**

Girdling of low-yield ‘Fortune’ mandarin on five of the six treatment dates, in both years, led to significantly increased yield. Late girdling, almost at the end of fruit drop, did not affect yield compared to the control (Figure 5A). Treatments at 15 DBA and 35 DAA were found to be the most effective at increasing fruit number, resulting in a 3.3-fold increase compared to the control (Figure 3).

Girdling of high-yielding ‘Fortune’ mandarin between A and 35 DAA, in both years, consistently increased yield compared to control trees. In both orchards, the effectiveness of girdling declined gradually as was delayed from 35 DAA until the end of fruit drop (Figure 5B). For both orchards, no interaction was observed between treatments and years.

In ‘Clausellina’, non-significant differences in fruit number and yield were found between trees girdled at 10 DAA and the controls, but girdling branches at 40 DAA significantly increased the number of fruits harvested and yield by 44% and 41%, respectively (Figure 6).

**DISCUSSION**

Abscission, as a self-thinning mechanism to adjust the number of fruits to the bearing potential of the tree, and its relation with fruit growth, are well-documented. Zuconi et al. (1978) observed that fruitlets stopped growing 7 d prior to abscission. Similarly, Agustí et al. (1982a) observed abscission of only the smallest fruits on ‘Navelate’ orange trees. Therefore, it was suggested that the lack of carbohydrates for developing fruitlets during competition between growing fruitlets, in a situation of profuse flowering, was the trigger for abscission (Goldschmidt and Monselise, 1977; Agustí et al., 1982a; Rivas et al., 2004). Furthermore, delayed abscission and increased fruit-set caused by girdling were associated with an increase in carbohydrate availability in ‘Shamouti’ orange (Monselise et al., 1972; Goldschmidt et al., 1985). Recent studies have shown that girdling affects the expression of genes related to starch accumulation (Li et al., 2003).

These data support the use of girdling as a cultural practice to improve yields in mandarin, as reported for

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**Table I**

<table>
<thead>
<tr>
<th>Girdling Treatment</th>
<th>Sucrose</th>
<th>Reducing Sugars</th>
<th>Fruit Set (%)</th>
<th>Final Fruit Set (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 DAA</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>13.7 c</td>
<td>7.6 a</td>
<td>13 c</td>
<td></td>
</tr>
<tr>
<td>15 DBA</td>
<td>21.1 b</td>
<td>6.7 a</td>
<td>46 b</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>33.4 a</td>
<td>6.5 a</td>
<td>60 a</td>
<td></td>
</tr>
<tr>
<td>50 DAA</td>
<td>10.5 a</td>
<td>5.2 a</td>
<td>3.5 c</td>
<td>0.2 c</td>
</tr>
</tbody>
</table>

\(1\) DBA, days before anthesis; A, anthesis; DAA, days after anthesis.

Results are expressed as mg g⁻¹ DW.

Data are means of 50 ovaries collected in the first year of Experiment 1. Means separation within columns was by Duncan’s New Multiple Range Test \((P \leq 0.05)\).
‘Clementina Fina’ (Del Rivero et al., 1969), ‘Oronules’ (Almela et al., 1993), ‘Ellendale’ tangor (Gravina et al., 1998), ‘Nova’ (Goren et al., 1992; Rivas et al., 2004), low yield ‘Shamouti’ orange (Monselise et al., 1972) and ‘Navelate’ sweet orange (Agustí et al., 1982b).

Our results show that the productivity of ‘Fortune’ and ‘Clausellina’ mandarins can also be improved by girdling. In ‘Fortune’, girdling was most effective when performed 15 DBA or 35 DAA, delaying abscission of young fruits and increasing the final fruit-set. This effect was reported previously by Monselise et al. (1972).

Examination of the carbohydrate contents of the developing fruitlets showed that the sucrose content was modified by the treatments. Sucrose levels increased a few days after girdling, enhancing its availability for metabolic process related to cell division during the early growth of fruitlets. Whereas flower formation may require only a threshold level of carbohydrates (Goldschmidt et al., 1985), fruit-set seems to be quantitatively correlated with carbohydrate availability (Schaffer et al., 1985; Goldschmidt and Huber, 1992; Fishler et al., 1983). The increased carbohydrate supply caused by girdling correlated with the transient reduction in fruitlet abscission that was observed. Similar findings were reported previously in ‘Ponkan’ mandarin by Mataa et al. (1998). The larger the number of fruitlets, the greater the amount of carbohydrate needed to maintain cell division and growth. For the low-yielding ‘Fortune’ mandarin, energy supply was sufficient for the first period of growth, but was insufficient for growth until 50 DAA. The higher fruit-set that resulted from girdling probably increased the demand for carbohydrate. It is possible that the tree could not support the higher demand from the growing fruitlets and competition between them triggered abscission (Iglesias et al., 2002). Furthermore, it seems that in low-yielding ‘Fortune’ mandarin, the carbon demand by fruitlets exceeded the photosynthetic capacity of the leaves, as reported by Moss et al. (1972). Thus, in the present study, by 50 DAA, levels of sucrose had

Fig. 4

Effect of girdling at 40 DAA on the time-course of accumulation of soluble carbohydrates [sucrose (Panel A); fructose (Panel B) and glucose (Panel C)] in ovaries of Satsuma cv. ‘Clausellina’ mandarin (Experiment 2). Data are the means of 50 ovaries ± SE. Statistically significant differences between treatments are given as *. Means separation was by Duncan’s New Multiple Range Test ($P \leq 0.05$).
Girdling effects on citrus fruit-set

decreased significantly in fruitlets of the girdling
treatments, because of the increase in fruit-set, and the
tree compensated for the lack of carbohydrates by
increasing fruitlet abscission. The control showed a
different abscission pattern, since it could maintain
almost constant levels of sucrose during fruitlet
development. Trees in the girdling treatments
maintained larger numbers of fruits and gave higher
yields than the controls.

In the highly productive ‘Clausellina’, the pattern of
fruit-set was different in terms of energy. At 50 DAA,
fruitlets borne on ‘Clausellina’ or ‘Fortune’ contained
similar concentrations of sucrose, although ‘Clausellina’
had higher fruit-set, which could be attributed to the high
concentration of reducing sugars. Once fruitlet
abscession decreased (about 50 DAA), soluble sugars
accumulated in the fruits, as described by Mehouachi
et al. (2000).

In conclusion, girdling branches increased fruit-set in
citrus. When girdling was performed close to anthesis, it
facilitated the setting process in a cultivar that is less
prone to set. When it was performed in full fruitlet
abscession (35–40 DAA), girdling facilitated the setting
process in both cultivars, irrespective of the ability of the
cultivar to set. In both cases, the increased availability of
carbohydrates to the fruitlets appears to be the most
plausible explanation for the increased fruit-set that
resulted from girdling.

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