

Girdling effects on fruit set and quantum yield efficiency of PSII in two *Citrus* cultivars

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Summary Girdling effects on fruitlet abscission, leaf chlorophyll, chlorophyll a fluorescence and carbohydrate concentration in various flowering and vegetative shoots were studied during natural fruit drop in two *Citrus* cultivars. Irrespective of shoot type, girdling delayed fruitlet abscission, but only fruitlets borne on leafy shoots had increased final fruit set. Chlorophyll a fluorescence analysis revealed differences in quantum yield efficiency of photosystem II of light adapted leaves (Φ_{PSII}) among shoot types and in response to girdling. In young leaves of vegetative shoots, girdling decreased Φ_{PSII} , whereas Φ_{PSII} increased from Day 30 after girdling in young leaves of leafy flowering shoots; however, Φ_{PSII} did not change in mature leaves during fruit set in either control or girdled trees. Girdling altered leaf carbohydrate concentrations and the photosynthetic performance of the various shoot types. Our results indicate that, in *Citrus*, several carbohydrate-based regulatory mechanisms of photosynthesis coexist during carbohydrate accumulation brought about by girdling. It is concluded that the delay in fruitlet abscission and the increase in Φ_{PSII} observed in girdled leafy flowering shoots are the mechanisms underlying the enhancement of fruit set after girdling.

Keywords: *abscission, carbohydrates, chlorophyll a fluorescence, sink activity, SPAD.*

Introduction

Fruit set in citrus requires large amounts of carbon compounds that are provided by current photosynthesis and reserves accumulated by the tree during winter (Goldschmidt and Koch 1996, Bustan and Goldschmidt 1998). Carbohydrate reserves are mainly used during the early stages of fruitlet development (Shimizu et al. 1978), when the requirement exceeds the capacity of the tree to synthesize carbohydrates (García-Luis et al. 1988, Bustan and Goldschmidt 1998). Afterward, as a consequence of sink competition, a decline in the amount of stored photoassimilates is reported to lead to fruitlet abscission (Goldschmidt and Monselise 1978, Rivas et al. 2006). During this stage, fruit survival depends mainly on carbohydrates sup-

plied by current photosynthesis (Iglesias et al. 2003). Consequently, during this stage, young leaves are assumed to be net carbohydrate exporters, i.e., from Day 20 to Day 25 after full bloom (Schaffer et al. 1985, Ruan 1993). Based on this sink-to-source transition, the ability of a citrus tree to set fruits has been related to the distribution of different shoot types (Agustí et al. 1982, Rivas et al. 2004). In particular, leafy flowering shoots maintain a higher frequency of fruit set than leafless flowering shoots (Moss 1970, Rivas et al. 2004). The fruit set process is complex because photosynthetic activity and, thus, the production of photosynthetic compounds, can be altered by sink demand. For instance, in many deciduous and evergreen fruit trees including citrus, high carbon requirements (e.g., during fruit development) result in an increase in photosynthetic rate (Iglesias et al. 2002, Syvertsen et al. 2003, Urban et al. 2004), but once carbon demands are fulfilled, any further increase in carbohydrate availability elicits end-product feedback control of photosynthetic activity as a result of the accumulation of carbohydrates (Azcon-Bieto 1983, Goldschmidt and Huber 1992, Iglesias et al. 2002, Urban et al. 2004).

Girdling performed during fruitlet drop enhances initial fruit set in *Citrus* (Rivas et al. 2006). This effect has been related to the accumulation of photoassimilates in the canopy as a result of the interruption of the downward transport of soluble sugars (Wallerstein et al. 1974). Because spring flush in *Citrus* gives rise to different types of shoots (leafless flowering shoots, leafy flowering shoots and purely vegetative shoots), we hypothesized that several carbohydrate-based regulatory mechanisms of photosynthesis coexist during the period of carbohydrate accumulation brought about by girdling. Current knowledge about how these mechanisms affect fruit set and the photosynthetic capacity of the various shoot types is sketchy.

Photosynthetic capacity during fruit development has previously been assessed by chlorophyll a (Chl a) analysis (Syvertsen et al. 2003, Urban et al. 2004). This technique permits rapid estimation of the quantum efficiency of electron transport through photosystem II (PSII) in leaves that can

then be related to CO₂ assimilation (Genty et al. 1989, 1990, Harbinson et al. 1990).

Our objectives were to determine the effects of trunk girdling on fruit set of leafless flowering shoots and leafy flowering shoots, and to examine how mature (8 to 12 months old) and young leaves (current spring flush) respond to the carbohydrate demands of developing fruitlets. We also studied the extent to which source leaves are able to modify the quantum yield efficiency of PSII (Φ_{PSII}) during fruit set in response to girdling.

Materials and methods

Plant material and growth conditions

The experiment was carried out on two cultivars of mandarin, grown either in containers or under field conditions. The container-grown trees were 2-year-old 'Loretina' mandarin (*Citrus reticulata* Blanco), grafted on Carrizo citrange (*Citrus sinensis* [L.] Osbeck \times *Poncirus trifoliata* Raf.). The trees were grown outdoors in Valencia, Spain (39° N), in 10-l plastic containers filled with a sandy-loamy soil and fertilized with N at 20 g tree⁻¹ year⁻¹. All trees were drip irrigated daily with sufficient water (1 liter tree⁻¹ day⁻¹) to maintain a minimum leaching fraction of 25%. Insects and diseases were monitored at 2-day intervals and controlled according to the recommendation of our entomology and phytopathology departments.

Field-grown trees were 7-year-old 'Nova' mandarin trees (*Citrus reticulata* Hort. ex Tan. \times (*Citrus paradisi* Macf. \times *C. tangerina* Hort. ex Tan.)), grafted on *Poncirus trifoliata* rootstock and grown in a commercial orchard on the southern coast of Uruguay (35° S). Trees were planted at a 5.5 \times 3 m spacing on a sandy-loamy soil, irrigated and fertilized with N at about 150 kg ha⁻¹ year⁻¹.

Ten trees of each cultivar were selected for similarity in size, vigor and flowering intensity, as described by Rivas et al. (2004). Briefly, flowering intensity was estimated by selecting four branches per tree with at least 600 nodes per branch. On each branch, sprouts were classified into five types: (1) single-flowering leafless shoots (SL) characterized by a single flower borne on the axis of a mature leaf (8 to 12-month-old); (2) multiple-flowering leafless shoots (ML) characterized by several flowers borne on the axis of a mature leaf; (3) single-flowering leafy shoot (SLY) characterized by a single flower borne in the apical position of a leafy shoot of the current spring flush; (4) multiple-flowering leafy shoots (MLY) characterized by several flowers borne on a leafy shoot of the current spring flush; and (5) vegetative shoots (VS) characterized by a flowerless leafy shoot of the current spring flush. For each branch, the number of nodes, the number of shoots of each type and the number of flowers and leaves of each shoot were recorded. From these data, we calculated: sprouts/100 nodes; flowers/100 nodes; number of each shoot type/100 nodes; mean number of flowers per shoot type; and mean number of leaves per shoot type.

Girdling treatment

For each cultivar, half of the trees were trunk girdled at anthesis (when 60% of the flowers were opened), 10 cm above the rootstock. A cut 1-mm wide was made completely encircling the trunk with a sharp hooked-blade. Care was taken to avoid injuring the xylem.

Fruit set and growth evaluation

Fifteen days before anthesis, the total number of flowers per tree (FPT) produced by the 2-year-old 'Loretina' mandarin trees was counted, and nets (9 m²) were spread under each tree. No flowers were shed before the commencement of the trial. Simultaneously, 10 reproductive shoots per tree of each type were tagged, and the numbers of flowers (NFS) and leaves per shoot were recorded. After girdling, fruitlets that dropped onto the nets (FDN) were collected and counted at weekly intervals (WI) until the end of natural fruitlet drop. Material collected at each interval was dried for 48 h at 60 °C and weighed. Abscission percentage at each date was calculated as: ((FDN_{WI}/FPT)100). On the same sampling dates as abscission analysis, persisting fruitlets per shoot (PFS) were counted and their equatorial diameters measured with a digital calliper. Fruit set per shoot type, on each measurement date, was calculated as: ((PFS_{WI}/NFS)100).

In 'Nova' trees, 15 days before anthesis, four branches with at least 800 nodes each were selected in all canopy orientations, and the number of flowers per branch (NFB) recorded. At the end of natural fruitlet drop, persisting fruitlets on each branch (PFB) were counted, and final fruit set was calculated as: ((PFB/NFB)100). Fruit set analysis per shoot was performed as described for 'Loretina' mandarin. In addition, in each shoot type, growth in fruitlet fresh mass was determined by harvesting 50 fruitlets per shoot at about 2-week intervals from non-experimental control and girdled trees of the same experimental plot.

Chlorophyll determination

Citrus leaf SPAD readings (SPAD-502; Minolta Co., Osaka, Japan) provide a nondestructive surrogate method for determining leaf chlorophyll (Chl) concentration (Jifon et al. 2005). To validate this procedure under our experimental conditions (growth environment and leaf anatomy), we calculated the regression equation between SPAD readings and Chl concentration for 'Loretina' mandarin. Twenty-five leaves having a wide range of greenness were selected. Leaves were chosen from all canopy orientations irrespective of the age of the branches. For each leaf, the mean of six SPAD readings (three on each side of the midrib) was recorded. All readings were conducted under natural light. Immediately after the SPAD readings, a sample (300 mg fresh mass) from the mid-lamina area was taken for Chl analysis. Chlorophyll was extracted with 3 ml of *N,N*-dimethylformamide for 48 h with constant stirring in the dark at 4 °C. Samples were centrifuged at 4000 g, and the absorbance of the supernatant determined at 647 and 664 nm with a spectrophotometer. Chl concentration was determined from the absorbance measurements as described by Moran

(1982), and its relationship with the SPAD reading established ($r^2 = 0.962$; $P \leq 0.01$). Based on the strong positive correlation between the SPAD readings and Chl concentrations in 'Loretina' mandarin, we used the regression equation to estimate leaf Chl concentration from SPAD measurements.

Experimental procedure

For each shoot type, five shoots per tree were selected for SPAD determinations. For leafy shoots, measurements were made on three young leaves (from the current-spring's flush). For leafless shoots, the data were collected from the mature leaf (8- to 12-month-old) just below the new shoot. Measurements were performed at the same time as the determinations of fruitlet abscission and fruit set.

Chlorophyll a fluorescence measurements

We determined the quantum yield efficiency of PSII by Chl a fluorescence analysis in the same leaves used for the SPAD reading. To ensure similar exposure to natural irradiance, shoots were selected from the external south-east side of the canopy at a height of about 1.3 m. Measurements were made with a pulse amplitude modulated system (Junior-PAM, Walz, Gademann Instrument, Germany) at about weekly intervals between anthesis until the end of natural fruitlet drop. Evaluations were initiated two weeks before girdling to ensure that there were no differences among trees at the beginning of the trial. Measurements were performed before midday (0830–1030 h). Before the measurements, leaves were dark-adapted for 30 min to obtain the maximum quantum yield of PSII (F_v/F_m , where F_v and F_m are variable and maximum fluorescence, respectively). The minimum Chl fluorescence (F_o) was assessed with a weak modulated pulse ($< 0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$), and F_m , corresponding to all PSII centers in the closed state, was induced by a 1-s pulse of saturating white light ($10,800 \mu\text{mol m}^{-2} \text{s}^{-1}$). Variable fluorescence was calculated as the difference between F_m and F_o . The value of quantum yield efficiency of PSII of light adapted leaves (Φ_{PSII}) was calculated according to the formula $\Phi_{\text{PSII}} = (F_m' - F_s)F_m'$, where F_m' and F_s are the maximum and steady-state fluorescence yield of light adapted leaves, respectively (Genty et al. 1989). The steady-state Chl fluorescence (F_s) was measured with an actinic light pulse ($270 \mu\text{mol m}^{-2} \text{s}^{-1}$), and F_m' was induced by a 1-s pulse of saturating white light.

Carbohydrate analysis

In a separate experiment, 25 shoots of each type (five shoots per tree) from each treatment were excised from 2-year-old potted 'Loretina' mandarin trees at 30 days after girdling (DAG). Each shoot was dissected into leaves, stem and in the case of MLY, also fruitlets. Samples were frozen immediately in liquid N_2 , lyophilized and stored as powders at -28°C . Soluble carbohydrates were extracted and purified as described by Rivas et al. (2006). Briefly, each sample (100 mg) was extracted three times with 1.0 ml of 80% (v/v) ethanol at 85°C , and 0.1 ml of fucose (60 mg ml^{-1}) was added as an internal standard. After centrifugation at 15,000 g, the pellet was re-

tained for starch determination, and the supernatant was evaporated to 0.5 ml in vacuo, and purified. Purification steps included sequential column chromatography on cation Dowex (pH 7.5) 50×80 –100 mesh and anion (pH 4.5) 1×40 mesh (Sigma Química), filtration through a $0.45 \mu\text{m}$ membrane (Waters-Millipore, Barcelona, Spain), and chromatography through a C18 Sep-Pak cartridge (Waters-Millipore). Each purified sample was dried in vacuo and redissolved in 60 μl of double-distilled water. Twenty μl aliquots were analyzed by high-performance liquid chromatography (HPLC) in a Spectra HPLC System[®] (California, USA) with an APS-2 Hypersil, $250 \times 4.6 \text{ mm}$ column attached to an IonGuard pre-column ($20 \times 0.65 \text{ mm}$) connected to a differential refractometer R150 vacuum pump P2000. The solvent was acetonitrile:water (6:4, v/v) applied at a flow rate of 1 ml min^{-1} for the 20-min run. Data were processed with the ChromQuest[®] software system. Sucrose, glucose and fructose were identified by their retention times. The recovery of sugars was evaluated by comparison with that of the fucose internal standard.

After the extraction of soluble sugars, starch concentration of the remaining pellet was determined. The pellet was dissolved in 6.0 ml of water, gelatinized by autoclaving for 2 h at 135°C , and centrifuged for 10 min at 27,000 g. Four ml of the supernatant were removed, and the samples were incubated for 2 h at 55°C with shaking, with 0.2 ml of fucose (60 mg ml^{-1}) as an internal standard, 0.5 ml of Na-acetate (pH 4.5) and 1 ml of amyloglucosidase (1218 units, from *Rhizopus* mold, Sigma). Samples were centrifuged for 30 min at 27,000 g, and the supernatant dried in vacuo and redissolved in 1 ml of double-distilled water. Released glucose was determined by HPLC as described previously.

Statistical analysis

The effects of girdling on fruit set, fruit growth and mass, Chl concentration, Chl a fluorescence and carbohydrate concentration were evaluated by analysis of variance (ANOVA), and comparisons of means were made by Duncan's multiple range test (DMRT). Percentages were analyzed after arc-sine transformation of the data. The relationship between SPAD readings and leaf Chl concentrations was evaluated by regression analysis. The experimental data were analyzed with Statgraphics 4.1 software (Statistical Graphics Corp.).

Results

Effects of girdling on fruit set in relation to shoot type and fruit growth

For both cultivars, girdling caused significant differences in fruit set between shoot types (Table 1). Although abscission pattern analysis revealed that girdling delayed fruitlet abscission regardless of shoot type, only leafy flowering shoots (SLY and MLY) showed improved final fruit set in response to girdling (Figures 1 and 2). In leafless flowering shoots on both girdled and non-girdled trees, almost all fruits had abscised by the end of the natural fruitlet drop period.

In 'Loretina' mandarin, abscission pattern analysis of

Table 1. Effects of girdling at anthesis on final fruit set in 'Loretina' and 'Nova' mandarin in relation to shoot type. For each shoot type, number of flowers or leaves or both, per shoot is given. Data are the means \pm SE of 50 shoots. Significant differences ($P \leq 0.05$) within the same shoot and cultivar are denoted by an asterisk (*). Abbreviations: SL = single-flowering leafless shoot; ML = multiple-flowering leafless shoot; SLY = single-flowering leafy shoot; MLY = multiple-flowering leafy shoot; (-) = control; and (+) = girdling.

Cultivar	Shoot type	Treatment	Flowers shoot ⁻¹	Leaves shoot ⁻¹	Fruit set (%)
'Loretina'	SL	-	1.0	—	0.0 \pm 0.0
	SL	+	1.0	—	0.0 \pm 0.0
	ML	-	3.1 \pm 0.3	—	1.3 \pm 0.8
	ML	+	3.0 \pm 0.3	—	2.7 \pm 1.1
	SLY	-	1.0	3.9 \pm 0.5	0.0 \pm 0.0 *
	SLY	+	1.0	4.2 \pm 0.4	12.0 \pm 3.7
	MLY	-	3.8 \pm 0.4	3.6 \pm 0.4	2.7 \pm 1.2 *
	MLY	+	4.0 \pm 0.4	3.4 \pm 0.5	14.4 \pm 1.7
'Nova'	SL	-	1.0	—	0.0 \pm 0.0
	SL	+	1.0	—	0.0 \pm 0.0
	ML	-	5.1 \pm 0.6	—	3.3 \pm 1.2
	ML	+	4.5 \pm 0.4	—	2.7 \pm 0.8
	SLY	-	1.0	3.9 \pm 0.4	20.0 \pm 3.2 *
	SLY	+	1.0	4.3 \pm 0.7	32.0 \pm 4.1
	MLY	-	4.8 \pm 0.8	4.3 \pm 0.4	8.7 \pm 2.8 *
	MLY	+	4.6 \pm 0.9	4.7 \pm 0.5	15.7 \pm 3.1

fruitlets on leafless and leafy single-flowering shoots (SL and SLY) revealed that fruitlets on SL and SLY shoots had higher abscission rates in control trees than in girdled trees (Figure 1). The effect of girdling was evident 7 to 28 DAG for fruitlets on SL shoots and 7 to 42 DAG for fruitlets on SLY shoots. In SL and SLY shoots of control trees, more than 80% of fruitlet abscission occurred during these intervals. By the end of fruitlet drop (70 DAG), fruit set was significantly higher

on SLY shoots in girdled trees than in control trees, whereas fruitlets on SL shoots shed completely in both control and girdled trees (Table 1). For leafless and leafy multiple-flowering shoots (ML and MLY, respectively), the effects of girdling became significant at 21 and 28 DAG, respectively (Figure 1). Fruitlets on ML and MLY shoots had a similar tendency to abscise, with the higher rates occurring between 21 and 49 DAG (Figure 1). Final fruit set in ML shoots was negligible in

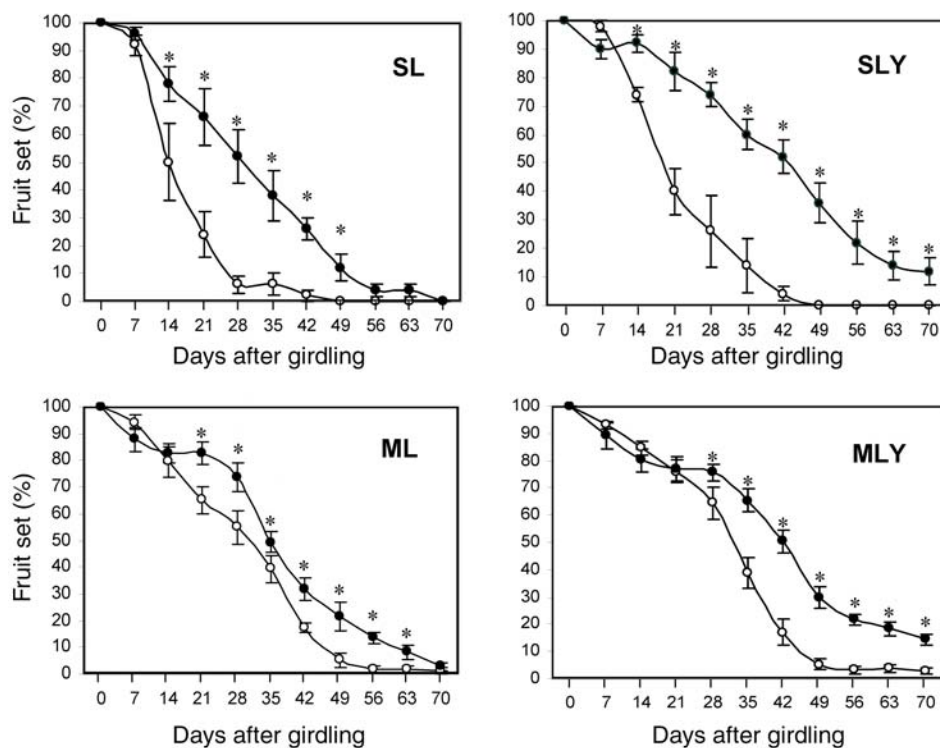


Figure 1. Fruit set pattern of the flowering shoots in 2-year-old 'Loretina' mandarin trees trunk girdled at anthesis (●) or ungirdled (○). Each value is the mean \pm SE of 50 shoots. For each shoot, significant differences ($P \leq 0.05$) between treatments are denoted by an asterisk (*). Abbreviations: SL = single-flowering leafless shoot; SLY = single-flowering leafy shoot; ML = multiple-flowering leafless shoot; and MLY = multiple-flowering leafy shoot.

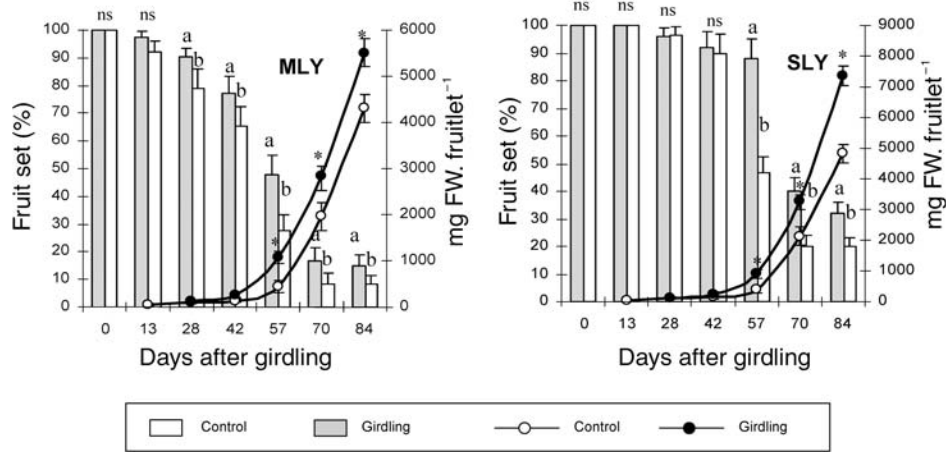


Figure 2. Effects of girdling at anthesis on fruit set (bars) and fruit growth (lines) patterns of multiple-flowering and single-flowering leafy shoots in 'Nova' mandarin. Fruit set values are means \pm SE of 50 shoots. Fruit growth measurements are means \pm SE of 50 fruits. Different letters above bars indicate significant differences ($P \leq 0.05$) in fruit set. Significant differences ($P \leq 0.05$) in fruit growth are denoted by an asterisk (*). Abbreviation: MLY = multiple-flowering leafy shoot; SLY = single-flowering leafy shoot; and ns = not significant differences found at $P \leq 0.05$.

both control and girdled trees. For MLY shoots, girdling significantly enhanced final fruit set in both cultivars (Table 1).

In 'Nova' mandarin, girdling increased fruit set and fruitlet mass on leafy flowering shoots (Figure 2; Table 1). For leafless flowering shoots, although girdling delayed fruitlet abscission (data not shown), it had no effect on final fruit set (Table 1). In 'Loretina' mandarin, from 42 DAG onward, both persisting and abscising fruits on girdled trees grew faster (Figure 3A) and were heavier (Figure 3B) than fruits on control trees.

In both cultivars, leafy shoots of both control and girdled trees did not differ either in number of leaves or number of flowers, nor was there any difference in the number of flowers on leafless shoots (Table 1). Therefore, the differences in fruit set and fruitlet mass cannot be attributed to differences in shoot characteristics.

Chlorophyll concentration and chlorophyll a fluorescence analysis

In mature leaves, there were no differences in Chl concentration or Φ_{PSII} (Figure 4) between control and girdled trees. These parameters remained almost constant throughout the

measurement period. The F_v/F_m ratio did not differ significantly from Φ_{PSII} , with values varying between 0.6 and 0.7 (data not shown).

Girdling had no effect on foliar Chl concentrations in leafy flowering shoots, but there was a significant increase ($P \leq 0.05$) in Φ_{PSII} in girdled trees from 30 DAG (Figure 5). Since young leaves had a lower Φ_{PSII} at 2 DAG than mature leaves (Figures 5 and 6), a sharper Φ_{PSII} time course of young leaves was apparent in comparison to mature leaves. As for mature leaves, no differences between F_v/F_m and Φ_{PSII} were observed between control and girdled trees (data not shown).

Girdling significantly reduced Φ_{PSII} in leaves of vegetative shoots (Figure 6). The reduction was apparent throughout most of the measurement period (from 7 to 61 DAG). Leaf Chl concentration in vegetative shoots showed a similar trend to that in leafy flowering shoots, and, similarly to the other shoot types, it was unaffected by girdling.

Leaf carbohydrate concentration

Significant differences in the concentration of leaf carbohydrates were found 30 DAG between treatments and shoot types (Table 2). In mature leaves, girdling did not significantly af-

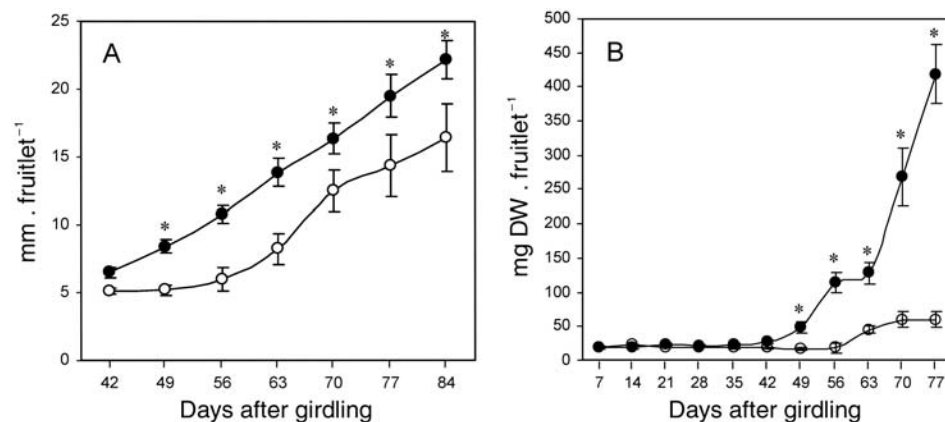


Figure 3. Diameter of persisting fruitlets (A) and dry weight of abscised fruitlets (B) of trunk girdled (●) or ungirdled (○) trees in 'Loretina' mandarin. Values are means \pm SE of at least 25 fruits tree⁻¹. Significant differences ($P \leq 0.05$) are denoted by an asterisk (*).

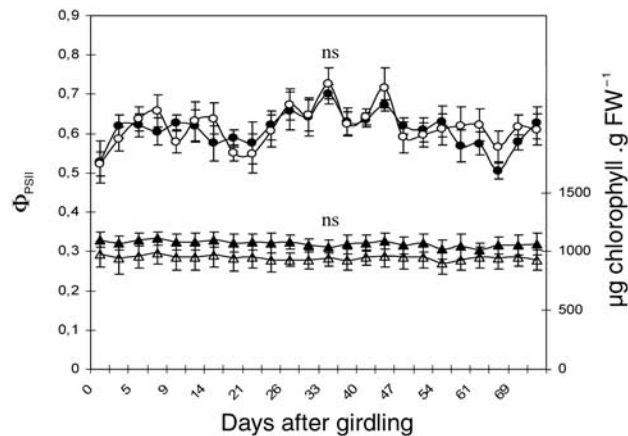


Figure 4. Quantum yield efficiency of photosystem II (Φ_{PSII} , \circ , \bullet) and chlorophyll concentration (Δ , \blacktriangle) of mature leaves of trunk girdled (filled symbols) and ungirdled (open symbols) 'Loretina' mandarin trees. Data are means \pm SE of 25 leaves. None of the treatment differences were significant at $P \leq 0.05$ (ns = not significant).

fect fructose and sucrose concentrations, but it increased glucose and starch concentrations by 20.8 and 116.6%, respectively. Among shoot types, vegetative shoots showed the greatest increases in leaf carbohydrate concentrations in response to girdling, with increases in fructose, glucose and starch concentrations of 111.9, 83.1 and 147.6%, respectively; however, girdling had no significant effect on foliar sucrose concentration of vegetative shoots. Compared with control trees, leaves on MLY shoots of girdled trees had increased concentrations of all carbohydrates, including over a 90% increase in sucrose concentration. Conversely, MLY shoots of girdled trees showed the lowest increase in foliar starch concentration among shoot types.

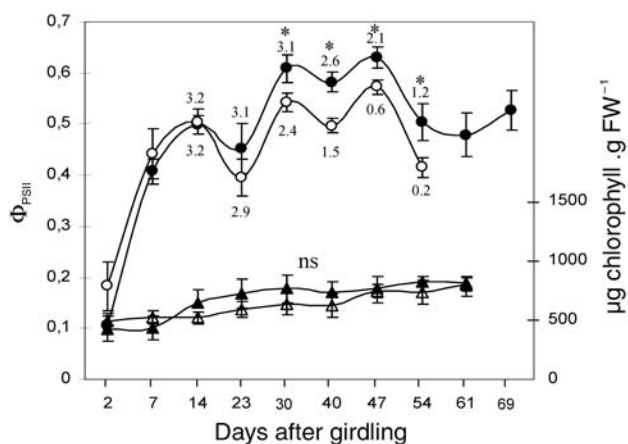


Figure 5. Quantum yield efficiency of photosystem II (Φ_{PSII} , \circ , \bullet) and chlorophyll concentration (Δ , \blacktriangle) of young leaves of multiple-flowering leafy shoots of trunk girdled (filled symbols) and ungirdled (open symbols) 'Loretina' mandarin. Data are means \pm SE of 25 leaves. Numbers in the figure are means of the number of fruitlets per shoot. Significant differences ($P \leq 0.05$) between treatments are denoted by an asterisk (*).

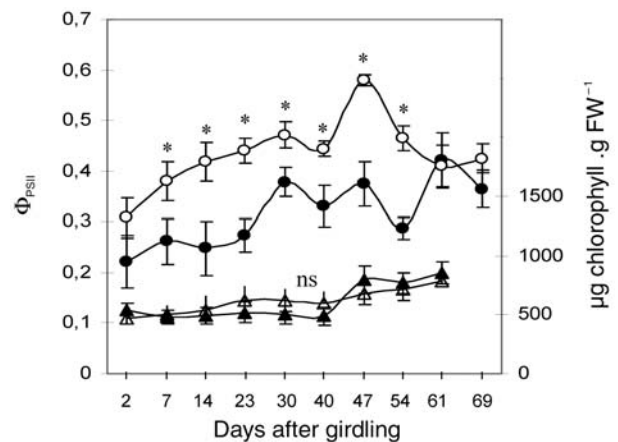


Figure 6. Quantum yield efficiency of photosystem II (Φ_{PSII} , \circ , \bullet) and chlorophyll concentration (Δ , \blacktriangle) of young leaves of vegetative shoots of trunk girdled (filled symbols) and ungirdled (open symbols) 'Loretina' mandarin. Data are mean \pm SE of 25 leaves. Significant differences ($P \leq 0.05$) between treatments are denoted by an asterisk (*).

Discussion

Our results demonstrate that girdling affects fruit set differently depending on shoot type. Although girdling delayed fruitlet abscission in all shoot types, only leafy flowering shoots (SLY and MLY) had increased final fruit set (Figures 1 and 2). In contrast, fruitlets arising from leafless flowering shoots shed almost completely in both cultivars (Table 1). Thus, our results demonstrate that the increase in fruit set in response to girdling is related to a selective process that only benefits fruitlets produced by leafy flowering shoots. Furthermore, Chl a fluorescence analysis revealed a difference in response of Φ_{PSII} to girdling between mature and young leaves of vegetative shoots (VS) and leafy flowering shoots (MLY).

Girdling had no effect on Φ_{PSII} in mature leaves and Φ_{PSII} remained more or less constant throughout the study (Figure 4). Although changes in Φ_{PSII} do not necessarily imply changes in net CO_2 assimilation, a constant Φ_{PSII} suggests that mature leaves did not manifest a feed-forward response to an increase in carbohydrate demand by nearby growing fruitlets, as has been proposed by others (Monselise and Lenz 1980, Syvertsen et al. 2003). Furthermore, during fruit set, there was a twofold increase in starch concentration in mature leaves whereas their sucrose concentration remained constant (Table 2). Starch accumulation even under conditions where fruitlet carbon demand is not fully assured has been reported previously (Fishler et al. 1983) and may represent a survival mechanism in *Citrus* (Goldschmidt and Koch, 1996). In both control and girdled trees, Φ_{PSII} did not differ from F_v/F_m (data not shown), indicating that the quantum yield efficiency of mature leaves remained at maximal values during the onset of fruit development. Consequently, because fruitlets developing on leafless flowering shoots rely solely on carbohydrates supplied from adjacent mature leaves (Erner 1989), the increase in initial fruit set that occurred in girdled trees must have been accompanied by an initial increase in carbohydrate availability

Table 2. Effects of girdling at anthesis on leaf carbohydrate concentrations ($\text{mg g}_{\text{DW}}^{-1}$) in 2-year-old 'Loretina' mandarin. Values correspond to 30 DAG. Each value is the mean of four independent analyses. Values in parenthesis denote the percentage increment over control trees. Significant differences at $P \leq 0.05$ and $P \leq 0.01$ are denoted by single (*) and double (**) asterisks, respectively. Means separation of $\text{mg g}_{\text{DW}}^{-1}$ by DMRT. Abbreviations: ML = mature leaf; VS = vegetative shoot; MLY = multiple-flowering leafy shoot; (-) = control; (+) = girdling; and ns = differences not significant at $P \leq 0.05$.

Sample	Treatment	$\text{mg g}_{\text{DW}}^{-1}$			
		Fructose	Glucose	Sucrose	Starch
ML	-	12.9	14.9	8.9	50.6
	+	13.7 (6.2)	18.0 (20.8)	12.2 (37.1)	109.6 (116.6)
	Significance	ns	*	ns	**
VS	-	6.7	24.8	10.2	34.9
	+	14.2 (111.9)	45.4 (83.1)	10.5 (2.9)	86.4 (147.6)
	Significance	**	**	ns	**
MLY	-	11.1	37.8	11.1	66.8
	+	15.1 (36.0)	49.3 (30.4)	21.4 (92.8)	78.4 (17.4)
	Significance	**	*	**	**

brought about by girdling (Rivas et al. 2006). Thereafter, however, fruitlets on leafless flowering shoots of girdled trees exhibited a high abscission rate and no differences in fruit set were observed between girdled and non-girdled trees by the end of the natural fruit drop period (Figure 1, Table 1).

Young leaves on leafy flowering shoots of girdled trees had significantly increased Φ_{PSII} from 30 DAG compared with control trees (Figure 5). This increase coincided with the sink-to-source transition. After this transition, control trees had a higher fruitlet abscission rate than girdled trees (Figures 1 and 5). Our results help clarify the source-sink interaction response. Thus, girdling promoted initial fruit set in all shoot types during the period of dependence on imported carbohydrate supply. Subsequently, during a period marked by an increase in carbohydrate production by young leaves, growing fruitlets exerted a feedforward effect on Φ_{PSII} . As the number and size of the growing fruitlets increased, young leaves, unlike the mature leaves, responded to the increasing carbohydrate requirement by increasing Φ_{PSII} (Figure 5). This effect has been observed previously in many deciduous and evergreen fruit trees including *Citrus* (Layne and Flore 1995, Wünsche et al. 2000, Syvertsen et al. 2003, Urban et al. 2004). Carbohydrate distribution in MLY leaves appeared to parallel the increase in Φ_{PSII} . Sucrose, which was the major transport form of carbon from leaves to the fruitlets, increased in young MLY leaves (Table 2). The lack of increase in starch concentration in young MLY leaves suggests that photosynthates were diverted to sucrose synthesis to satisfy the carbohydrate requirements of the growing fruitlets (Table 2). Furthermore, the sucrose concentration in the stem phloem of MLY shoot increased by 47% in girdled trees 30 DAG (data not shown). Because fruit set in this period was higher in girdled trees than in non-girdled trees (Figure 1), the increase in stem phloem sucrose concentrations can be attributed to increased sucrose transport from leaves to fruitlets in response to a sink carbon demand. The time course oscillations observed in Φ_{PSII} in MLY leaves could be evidence of pulses of production and

transport of soluble carbohydrates, perhaps indicating that sustained high concentrations of soluble carbohydrates are incompatible with high rates of photosynthesis in young leaves (Sheen 1990).

Unlike MLY shoots, girdling significantly reduced Φ_{PSII} in vegetative shoots (Figure 6). This could be related to the increase in carbohydrate concentration in response to girdling and a lack of sink activity in these shoots causing end-product feedback inhibition of photosynthesis, as is well-documented for many crop species (Harrel and Williams 1987, Goldschmidt and Huber 1992, Iglesias et al. 2002, Urban et al. 2004). This idea is supported by the finding that girdling increased foliar glucose and starch concentrations of vegetative shoots (Table 2), which suggests that glucose in vegetative shoots, unlike MLY shoots, is diverted to starch synthesis thereby mediating the putative feedback response caused by the low demand for carbohydrates.

The feed-forward mechanism may be triggered by an enhancement of sink utilization rate of sugars, releasing cytosolic inorganic phosphate (Pi) in source leaves, promoting photophosphorylation and increasing CO_2 fixation to triose-P by Rubisco (Leegood, 1996; Paul and Pellny, 2003). It has been suggested that maximal photosynthetic rates cannot be maintained by Pi-cycling via stromal starch synthesis alone, and the chloroplast is often considered to be dependent on cytosolic sucrose synthesis for its Pi supply (Foyer and Galtier, 1996). Consequently, the extent of feedback modulation of photosynthesis depends on the nature of the final synthesized carbohydrate. Furthermore, when sucrose is the main storage carbohydrate, the leaves appear to show smaller decreases in CO_2 fixation than leaves that accumulate starch (Goldschmidt and Huber 1992). Therefore, the differences in Φ_{PSII} between MLY and VS could be explained by differences in the synthesis of the end-products of photosynthesis in response to a sink demand.

The involvement of a hormonal control of photosynthesis by the fruit is possible, because GA_3 sprays have been shown

to increase photosynthetic rate by promoting Rubisco activity (Yuan and Xu 2001). Furthermore, increased GA₁ concentrations were found in developing fruitlets of girdled trees (Talón et al. 2000). Therefore, GA mediated-signals from growing fruitlets could elicit the feed-forward response observed in our experiment. This hypothesis is supported by the finding that girdled trees showed increased fruitlet growth from Day 42 after girdling (Figures 2 and 3). The timing of this girdling response coincides with the increase in the GA concentration of fruitlets between Day 40 and 70 after girdling, reported by Talón et al. (2000).

Our results suggest that, irrespective of shoot type, young leaves but not mature leaves, are able to modulate Φ_{PSII} in response to carbohydrate demands during natural fruitlet drop. Our results confirm the hypothesis that, in response to girdling, different carbohydrate-based regulatory mechanisms of photosynthesis coexist in different citrus shoot types, as observed for VS and MLY. These mechanisms seem to modulate Φ_{PSII} thereby adjusting source/sink balances to optimize carbon economy during fruit set. We demonstrated that Φ_{PSII} is involved in at least one step of the metabolic coordination between source and sink during fruit set. Furthermore, comparisons between VS and MLY indicated that this control may operate at an early stage of leaf development, even before the sink-to-source transition. The reason why mature leaves actively accumulated starch and showed no change in Φ_{PSII} during fruitlet drop remains unclear and deserves further study.

In conclusion, girdling increased final fruit set only in leafy shoots. We demonstrated that the delay in fruitlet abscission and the increase in Φ_{PSII} in girdled leafy flowering shoots were the mechanisms underlying the enhancement of fruit set by girdling. Because mature leaves did not adjust Φ_{PSII} and vegetative shoots reduced Φ_{PSII} in response to girdling, mature leaves and vegetative shoots support the fruit set process, but they are not implicated in the improvement in fruit set that results from girdling.

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