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Carbon utilization by fruit limits shoot growth in alternate-bearing citrus trees

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ABSTRACT

Fruit load in alternate-bearing citrus trees is reported to alter shoot number and growth during spring, summer, and autumn flushes, and the source–sink balance, which affects the storage and mobilization of reserve nutrients. The aim of this work was to assess the extent of shoot growth inhibition resulting from the presence of fruits in 'Moncada' mandarin trees loaded with fruit (ON) or with very light fruit load (OFF), and to identify the role of carbohydrates and nitrogenous compounds in the competition between fruits and shoots. Growth of reproductive and vegetative organs was measured on a monthly basis. ^{13}C- and ^{15}N-labeled compounds were supplied to trace the allocation of reserve nutrients and subsequent translocation from source to sink. At the end of the year, OFF trees produced more abundant flushes (2.4- and 4.9-fold higher in number and biomass, respectively) than ON trees. Fruits from ON trees accumulated higher C amounts at the expense of developing flushes, whereas OFF trees exhibited the opposite pattern. An inverse relationship was identified between the amount of C utilized by fruits and vegetative flush growth. ^{13}C-labeling revealed an important role for mature leaves of fruit-bearing branches in supporting shoot/fruit growth, and the elevated sink strength of growing fruits on shoots. N availability for vegetative shoots was not affected by the presence or absence of fruits, which accumulated important amounts of ^{15}N. In conclusion, our results show that shoot growth is resource-limited as a consequence of fruit development, and vegetative-growth inhibition is caused by photoassimilate limitation. The competence for N is not a decisive factor in limiting vegetative growth under the experimental conditions of this study.

Introduction

Alternate bearing is characteristic of some citrus cultivars, including many late-ripening mandarins, in which a heavy crop (ON year) is followed by a very light crop (OFF year) in the next year. Fruit-induced inhibition of flowering is the primary cause of alternate bearing (Moss, 1971), and early removal of fruit counteracts this effect (Goldschmidt and Golomb, 1982; García-Luis et al., 1986; Martínez-Fuentes et al., 2010). The extent of this inhibition is determined by factors such as cultivar, environmental conditions, number of fruits per tree, and harvesting date (Monselise and Goldschmidt, 1982). Thus, trees that flower copiously and produce a heavy crop load tend to form very few flowers in the next year (Schaffer et al., 1985).

At molecular level, important homologous genes involved in flowering have been isolated and characterized, including *FLOWERING LOCUS T* (Endo et al., 2005), *TERMINAL FLOWER*, *LEAFY* and *APETALA1* (Pillitteri et al., 2004a,b), *APETALA3*, *SUPRESSOR OF OVEREXPRESSION OF CONSTANS1*, and *WUSCHEL* (Tan and Swain, 2007). It has been proposed that these genes constitute a complex network regulating flowering (Dornelas et al., 2007a,b). The ectopic expression of a citrus *Flowering Locus T gene* (*CiFT*) can induce extremely early flowering in *Poncirus trifoliata* (Endo et al., 2005). The expression of *CiFT* homologs show a seasonal increase during the floral-induction period, and were enhanced by an artificial low-temperature treatment (Nishikawa et al., 2007). These results indicate that *Citrus FT* homologs play a crucial role in floral induction. A study of the molecular basis of floral regulation by crop load reports that fruits repress *CiFT* expression in 'Moncada' mandarin, suggesting that this gene also plays a pivotal role in the alternate-bearing process (Muñoz-Fambuena et al., 2011, 2012).

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It has been proposed that fruits may influence flowering by reducing carbohydrate reserves (Ogaki et al., 1963; Goldschmidt and Golomb, 1982). This hypothesis is supported by the observation that girdling in alternate-bearing citrus trees increases carbohydrate levels in affected branches (Schaffer et al., 1985; Goldschmidt et al., 1985; García-Luis et al., 1995; Li et al., 2003), and these subsequently flower more profusely (Goldschmidt et al., 1985; Agustí et al., 1992). However, no consistent correlation has been found between carbohydrate levels and flowering in *Citrus* (Goldschmidt et al., 1985; García-Luis et al., 1988; Martínez-Fuentes et al., 2010), peach (Reig et al., 2006) or olive (Stutte and Martin, 1986) trees. A recent proteomic analysis of leaves and floral buds from ON and OFF trees at the time of floral induction showed that the largest group of proteins up-expressed in organs from OFF trees are enzymes involved in carbohydrate and nitrogen metabolism, indicating the importance of C and N compounds for flowering (Muñoz-Fambuena et al., 2013a,b).

Two additional effects have been associated with alternate bearing. The first is that the presence of fruits reduces vegetative development (Monselise and Goldschmidt, 1982). In alternate-bearing citrus trees, heavy fruit yield inhibits shoot number and growth during the three main flushes (spring, summer, and autumn), whereas low fruit yield (even when fruits are artificially removed during early development) restores vegetative growth (Verreynne and Lovatt, 2009; Martínez-Fuentes et al., 2010). The second effect is that differences in fruit load alter source–sink balances and affect the storage or mobilization of reserves. Consequently, the status and availability of C or N depends on the crop year (Lewis et al., 1964; Monselise et al., 1983). In alternate-bearing citrus trees, carbohydrate levels (soluble sugars and starch) in leaves, branches, and roots are lower at the end of ON years than at the end of OFF years (Jones et al., 1975; Goldschmidt and Golomb, 1982). Considered together, these two effects suggest that vegetative and reproductive organs compete for available nutrient reserves.

The aim of this work is to elucidate the extent to which shoot growth is inhibited by the presence of fruits, and to determine if growth reduction is due to a limitation of carbohydrate or nitrogenous compounds. We measured the growth of reproductive and vegetative organs in adult 'Moncada' mandarin trees loaded with fruit (ON) or with very low fruit yield (OFF). To understand the relationship between fruit consumption of carbon or nitrogen and the availability of these nutrients for new shoots, we analyzed C and N partition to developing shoots and fruits at monthly intervals during the year. Moreover, ON and OFF trees were supplied with ^{13}C- or ^{15}N-labeled compounds to trace their translocation from source to sink (developing fruits or new growing shoots) organs to assess nutrient competition between fruit and shoot. These experiments enable testing of the hypothesis that fruit reduces shoot growth because of competition between fruit and shoot for photoassimilates or nitrogenous compounds.

Materials and methods

Plant material and experimental design

Adult 'Moncada' mandarin trees [Clementina 'Oroval' (*Citrus clementina* Hort. ex Tan.) × 'Kara' mandarin (*Citrus unshiu* (Swingle) Marcow. × *Citrus nobilis* Lour.] budded on Carrizo citrange [*Citrus sinensis* Osbeck × *Poncirus trifoliata* (L.) Raf.] rootstock and growing under field conditions were used in this study ($n = 30$). Trees were spaced 5 × 5 m. The soil was sandy loam (Cambic Arenosol) with 26.4% $CaCO_3$ and pH 7.8. The grove was cultivated, pruned, and sprayed according to local practices, and the soil was kept free of weeds using post-emergence herbicides. Plants were watered using a drip-irrigation system with four emitters per tree, and the amount of water applied to each tree was equivalent to the total seasonal crop evapotranspiration (ETc; Doorenbos and Pruitt, 1977). The volume of water applied weekly was calculated using the following expression: ETc = ETo × Kc; where ETo is the reference crop evapotranspiration under standard conditions, determined using the Penman–Monteith approach (Allen et al., 1998), and Kc (crop coefficient) is a function of canopy size and leaf properties based on the guidelines provided by Castel and Buj (1994). The soil water potential was controlled daily using a ThetaProbe PR2 (Delta-T Devices, Cambridge, UK), and irrigation was scheduled when the matric potential at 30 cm depth attained −10 kPa. The fertilizer rate was based on tree canopy size. Nitrogen was annually supplied in the form of 700 g urea, 400 g potassium nitrate, and 200 g phosphoric acid per tree. The fertilizers-N were dissolved in the irrigation solution (fertigation) and split uniformly into 66 applications between March and October. The number of flowers was determined at full bloom (April), by counting the number of flowers within a 0.5 × 0.5 m frame, which was located at the canopy surface. The tree canopy was divided into four cardinal points and two heights (up and down-half of it) and a count was performed in each position (eight counts per tree), considering all flowers within the limits of the frame. The total number of flowers, on a tree basis, was estimated by extrapolating the mean number of flowers per frame to the total tree surface area. At the beginning of the experiment (April 2011), tree height (h) and canopy radius (r = average radius in the N, S, E, and W directions) were measured. The canopy surface area (m^2) was calculated according to the following formula of Serfontein and Catling (1968), assuming the shape of a prolate spheroid: $SA = \pi r^2 + \pi(hr/f)\cdot\sin^{-1} f$; $f = (1 - r^2/h^2)^{1/2}$. The same procedure was used to count the number of new vegetative shoots when reached their final size in June, September, and December, coinciding with the end of spring, summer, and fall flushes, respectively.

To perform the experiments, the ON–OFF cycle of half of the available trees was desynchronized by removing fruits in July three years earlier. During 2011, samples of developing shoots (corresponding to the spring, summer, and autumn flushes) and flowers or fruits were randomly collected at the end of each month ($n = 20$ per tree and sampling) during the growth period. Shoots were individually excised from the branch using precision scissors, and flowers were also removed. To quantify total biomass and C and N content of abscised organs, tree litter (flowers, petals, calyces, and fruitlets) was caught in nets throughout the experimental period. All samples were washed in nonionic detergent solution and then rinsed in distilled water several times. Organs were pooled into three groups, weighed, dried in a forced-draft oven at 65 °C for 48 h, and reweighed to determine the growth curves expressed on a dry weight (DW) basis. Then, samples were ground with a laboratory ball mill (Retsch MM301, Haan, Germany) until they could pass through a sieve with 0.3-mm diameter holes, and were stored until further analysis. Fruit drop from bloom to the end of the fruit-set period (90 days after anthesis) was periodically recorded (at monthly intervals) on a population of 300 tagged fruitlets (50 per tree). Cumulative abscission was expressed as a percent of total fruitlets dropped at each time with respect to total fruitlets tagged. In December 2011, the yield of each tree was harvested and weighed.

$^{13}CO_2$ pulse-labeling and sampling

^{13}C translocation from $^{13}CO_2$-labeled mature leaves to new shoots and fruitlets was determined in spring and summer flushes (April 18 and August 29, respectively) from ON and OFF trees. Fruit-bearing and nonbearing branches from ON and OFF trees, respectively, were used for ^{13}C labeling. Selected branches were