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Productivity of *Citrus latifolia* plants subjected to branch girdling

Alainy Carla de S. Nascente, Rosane Angelica R. dos Anjos, Priscilla Gomes de F. Santos, Leonardo Willian A. Mendes, José Paulo C. Custódio, Hilton Dion T. Júnior, Patrícia S. da Silveira and Fábio S. Matos*

Department of Plant Production, State University of Goiás, Brazil.

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Abscission of flowers and fruit in *Citrus latifolia* cultivars is more intense than in seeded *Citrus* ones. The purpose of this study was to evaluate the effect of different levels of branch girdling in *C. latifolia* fruit fixation. The work was carried out in an orchard of *C. latifolia* grafted onto three-year-old *Citrus limonia* spaced 3x1, located in the Goiás State University experimental field. The experiment was set up according to the randomized block design with four treatments (girdling of 0, 25, 50 and 100% of the branches existing in the plant), five replications, parcels of four usable plants and total of 20 plants. Girdling was accomplished on 29/08/2014 and full flowering occurred on 13/09/2014. The plants that had 100% of their branches girdled presented high productivity of fruit with low juice volume. Those with 50% of branches girdled showed higher productivity and good juice yield per fruit. Results indicate that total blocking of transportation of assimilation from the canopy to the root system has negative impact on the volume of fruit juice.

Key words: Tahiti lime, carbohydrates, citrus culture, photoassimilates.

INTRODUCTION

The name 'lime' is used to refer to citrus fruit with highly acidic juice. Available statistics do not make a distinction between lemon and acid lime. However, estimates are that 70% of the world's total production consists of lemons and 30% of acid limes. The world's most produced and consumed type of acid lime is *Citrus latifolia* (Embrapa, 2015). Brazil is the world's greatest producer of sweet oranges and the fourth major producer of lemons, ranking only after Mexico, India and Argentina. Citrus culture holds key importance in Brazil's economy as it generates an annual contribution of about 5.2 million dollars to GNP. In the country, São Paulo state is the main producer of *C. latifolia* (Lopes et al., 2011; Soares et al., 2015).

The *C. latifolia* species is original from tropical regions, but the exact place of origin is still unknown. It is accepted, though, that is comes from citrus fruit seeds imported from Tahiti (Coelho, 1993). *C. latifolia* belongs

*Corresponding author. E-mail: Fabio.agronomia@hotmail.com.

in the Rutaceae family, with size ranging from medium to large, reaching up to 4 meters high green lanceolate mature leaves, and thin-peeled oblong oval fruit with smooth greenish to yellowish surface and maturing time (from anthesis up to harvesting) of around 170 days. It is a precocious culture with nearly year-round flower blossoming, though with higher concentration of flowers during the months of September and October (Rocha, 2008).

Long blossoming period coupled with high number of flowers and intense flower and fruit abscission are the main barriers to achieving great productivity of *C. latifolia* plants. Flower and fruit abscission in this species is higher than in seeded *Citrus* cultivars. Final fixation is barely around 1.85% in the September blossoming (Spósito and Mourão Filho, 2003). The factors leading to flower fall are still unknown, though they are assumed to be mostly physiologic and related to plant phenology as a result of competition for metabolites (Junqueira, 2013; Santos et al., 2014). According to Rivas et al. (2007), intensive blooming disturbs the partitioning of assimilates and a hormonal signal activates the abscission process to balance out the availability of carbohydrates and the fruit load to come. However, even after part of the flowers have fallen, significant fruit abscission occurs in the initial growth stage.

Adoption of management practices is required in order to minimize the abscission of reproduction structures and increase the productivity of *C. latifolia*. Among such practices branch girdling stands out, which consists in cutting off and removing a strip of bark around the trunk circumference (Pereira et al., 2010). Girdling prevents assimilation from being transported to the root system, increasing temporary retention of carbohydrates in the tree canopy and thus promoting greater fruit fixation (Santos et al., 2014). According to Pereira et al. (2014), girdling increases the number and size of *C. latifolia* fruit. Despite the benefits of girdling, the incision into the stem may cause accumulation of phytoregulators and other metabolites, bringing undesired effects such as reduced plant size, lower photosynthesis rate, yellowing leaves and reduced root growth (Pereira et al., 2010, 2011).

Inconsistent results obtained with girdling of *C. latifolia* and the effects of the incision on the plant's vegetative growth point to the need for research, in order to better understand the physiological aspects involved and thus justify the adoption of this agricultural practice. Therefore, this study was aimed at evaluating the effect of girdling of different amounts of branches on the fixation of *C. latifolia* fruit.

MATERIALS AND METHODS

The work was carried out in an orchard of *C. latifolia* grafted onto three-year-old *Citrus limonia* spaced 3x1 located in Goiás State University experimental field, Ipameri Campus (17°43'19''S, 48°09'35''W, Alt. 773 m). According to Köppen classification, the region has tropical climate with dry winter and rainy summer (Aw). There are two well-defined seasons: rain from October to April and drought from May to September. The soil of the experimental area is red-yellow Oxisol.

The experiment was set up following the randomized block design with four treatments (girdling of 0, 25, 50 and 100% of the number of branches existing in the), five replications, parcels of four usable plants and total of 20 plants. The girdling was accomplished by cutting into the phloem all around the stem circumference without damaging the xylem. The procedure was done with a jackknife that made it possible to remove a 2 mm-thick ring from the bark. Girdling was carried out on 29/08/2014 and full blooming occurred on 13/09/2014. Evaluations regarding the vegetative growth (plant height and stem diameter) were made on the day of girdling and 150 days thereafter (two times). Total foliar concentrations of chlorophylls and carotenoids were measured 60 days after girdling, and productivity variables (fruit diameter and weight, juice volume, total acidity, juice pH and productivity) were recorded during harvest.

Growth and reproduction variables

Plant height and stem, canopy and fruit diameter were measured using a graded ruler and a digital pachymeter. The number of fruits was determined by counting, and the mass was weighed with digital scales. We use the minimum diameter of 40 mm as a reference for the harvesting of fruits.

Determining juice total acidity, volume and pH

The juice volume was determined with a manual extractor and a beaker for precision measurement of four fruits per plant. After the juice was extracted, 10 ml of it were pipetted into an Erlenmeyer flask and distilled water was added to bring the volume to 60 ml. Subsequently, four drops of 1% phenolftalene solution were added and titrated with 0.1 M sodium hydroxide solution until pink color was reached. Calculation of total acidity was made through the equations proposed by Adolfo Lutz Institute (2008). Juice pH was determined with a previously Tri-Meter calibrated digital pH meter.

Photosynthetic pigments

In order to determine the concentration of chlorophylls were removed with two discs 1.2 cm in diameter each on fully expanded leaves located between the 3rd and 4th leaf pair stems and placed in glasses containing dimethyl sulfoxide (DMSO). Next, extraction was performed in water bath at 65°C for four hours. Aliquots were extracted for spectrophotometric reading at 480, 649.1 and 665.1 nm. The chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) contents were determined through the equation proposed by Wellburn (1994).

Statistical procedures

The experiment was set up according to the randomized blocks design with four treatments (girdling of 0, 25, 50 and 100% of the branches existing in the plant), five replications and parcels of two usable plants. The variables were submitted to one-way ANOVA and Newman-Keuls test at 5% probability, using statistical software SISVAR (Ferreira, 2011).

Table 1. Minimum and maximum values, average, standard deviation, coefficient of variation (CV) and Shapiro-Wilks normality test (SK) of variables plant height and diameter (mm/month), number of fruits per plant, productivity (t ha^{-1}), fruit diameter (mm), total acidity (%), pH, juice volume (ml), total carotenoids (Car) and total chlorophylls (total Chl) in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Variable	Minimum	Maximum	Average	DV	CV (%)	SW
Height	4.5	23.25	13.8	4.83	43.05	0.96^{ns}
Diameter	0.75	2.67	1.56	0.53	34.65	0.98^{ns}
No. of fruit	274.0	1192.0	692.9	249.1	35.9	0.94^{ns}
Productivity	2.46	8.38	5.12	1.60	31.37	0.91^{ns}
Fruit diameter	40.6	50.25	46.5	2.65	5.70	0.97^{ns}
Total acidity	0.50	0.95	3.78	0.03	18.65	0.94^{ns}
pH	3.72	3.86	3.78	26.23	0.86	0.94^{ns}
Juice volume	35.0	134.0	77.0	26.23	33.73	0.95^{ns}
Car.	0.18	0.65	0.44	0.12	29.10	0.90^{ns}
Total Chl.	0.81	2.89	1.62	0.52	32.51	0.85^{ns}

Table 2. Variance analysis and means comparison of plant height, diameter (mm/month), number of fruits per plant, productivity (t ha^{-1}), and fruit diameter (mm) in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Source of variation	GL	Mean squares				
		Height (cm/month)	Stem diameter (mm/month)	N° fruits	Prod. (t ha^{-1})	Fruit diam. (mm)
Treatment	3	179.11*	0.68^{ns}	889818.4**	32.87**	8.84^{ns}
Residue	12	77.68	1.61	250748	18.30	148.46
CV (%)		18.72	26.13	18.49	21.05	6.54
Treatment		**Averages**				
0		16.25^{b}	2.02^{a}	638.6^{b}	3.43^{c}	46.64^{a}
25%		21.00^{a}	1.25^{a}	382.2^{c}	4.72^{bc}	45.69^{a}
50%		14.37^{b}	1.45^{a}	734.2^{b}	6.69^{a}	47.67^{a}
100%		6.25^{c}	1.86^{a}	971.4^{a}	6.12^{ab}	46.44^{a}

*significant at 5% probability; **significant at 1% probability; ns = non-significant by F test. Averages followed by the same letter within the column do not differ by Newman-Keuls test.

RESULTS

Analysis of the results presented significant data variability, as shown in Table 1. The treatments promoted considerable changes in vegetative growth for plant height and stem diameter, which increased by 81 and 71% when comparing the lowest and highest values obtained, respectively. The same comparison of number of fruits, productivity, juice volume and total chlorophylls showed variations of 77, 71, 74 and 72%, respectively.

Variance analysis and means comparison of plant height, stem diameter, number of fruits per plant, fruit productivity and diameter are shown in Table 2. Variables of stem diameter and fruit diameter did not present significant variations at 5% probability. Plant height increased significantly among the different treatments. Monthly increase in height was 70% lower in plants with 100% of branches girdled compared to plants with 25% of branches girdled. The number of fruits per plant was on average 60% higher in plants with 100% of branches girdled compared to plants with 25% of branches girdled, whereas the control treatment had intermediate value. Productivity was greater in girdled plants compared to the control. The average productivity of girdled plants was 41% greater than that of the non-girdled control.

Variance analysis and means comparison of total acidity, pH, juice volume per fruit, total carotenoids and chlorophylls are shown in Table 3. Variables of total acidity, pH, and total carotenoids and chlorophylls did not present significant variations at 5% probability. Juice volume per fruit was on average 40% greater in plants with 25% of branches girdled compared to plants with 100% of branches girdled. The control treatment presented intermediate values.

Table 3. Variance analysis and means comparison of total acidity (%), pH, juice volume (ml), total carotenoids (Car), and total chlorophylls (Chl) in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Source of variation	GL	Mean squares				
		Total acidity (%)	pH	Juice volume (ml fruit^{-1})	Car (mg g^{-1})	Total Chl (mg g^{-1})
Treatment	3	0.107^{ns}	0.06^{ns}	3052.4*	0.06^{ns}	1.07^{ns}
Residue	12	0.191	0.014	6427.5^{ns}	0.07^{ns}	2.40^{ns}
CV (%)		16.49	0.82	28.31	24.68	32.23
Treatment		**Averages**				
0		0.66^{a}	3.76^{a}	20.75^{a}	0.37^{a}	1.98^{a}
25%		0.80^{a}	3.81^{a}	21.95^{a}	0.34^{a}	1.52^{a}
50%		0.59^{a}	3.79^{a}	19.00^{a}	0.29^{a}	1.21^{a}
100%		0.72^{a}	3.79^{a}	13.18^{b}	0.50^{a}	1.98^{a}

* = significant at 5% probability; ** = significant at 1% probability; ns = non-significant by F test. Averages followed by the same letter within the column do not differ by Newman-Keuls test.

Table 4. Pearson correlation coefficients for stem diameter, productivity, fruit diameter, number of fruits, total acidity, pH, juice volume per fruit, total carotenoids, and total chlorophylls in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Variables	Stem diam.	Prod.	Fruit diam.	No. of fruits	Total acidity	pH	Juice volume	Total car.	Total chl.
Height	-0.93^{ns}	-0.25^{ns}	0.89^{ns}	-0.93^{ns}	0.76^{ns}	-0.58^{ns}	0.94*	-0.83^{ns}	-0.77^{ns}
Stem diameter		0.02^{ns}	-0.85^{ns}	0.90^{ns}	-0.72^{ns}	0.34^{ns}	-0.90^{ns}	0.83^{ns}	0.70^{ns}
Product.			0.12^{ns}	0.44^{ns}	-0.67^{ns}	-0.09^{ns}	-0.44^{ns}	0.52^{ns}	0.23^{ns}
Fruit diameter				-0.69^{ns}	0.40^{ns}	-0.74^{ns}	0.70^{ns}	-0.52^{ns}	-0.96*
No. of fruits					-0.93^{ns}	0.31^{ns}	-0.99**	0.97*	0.50^{ns}
Total acidity						-0.08^{ns}	0.93^{ns}	-0.98**	-0.18^{ns}
pH							0.33^{ns}	0.11^{ns}	0.83^{ns}
Juice volume								-0.97*	-0.51^{ns}
Car									0.30^{ns}

ns = non-significant; * = significant at 5%; ** = significant at 1%.

The Pearson correlation for all variables analyzed is shown in Table 4. High and positive correlations were found between the number of fruits per plant and total carotenoids, plant height and juice volume, total acidity and carotenoids, and juice volume and carotenoids. Significant high and negative correlations were observed between fruit diameter and total chlorophylls and number of fruits per plant and juice volume per fruit.

DISCUSSION

The adoption of practices to reduce the abscission of fruit and increase productivity represents an important alternative to raise profitability for *C. latifólia* producers. Overall, branch girdling caused significant changes to vegetative growth and fruit yield, as discussed below.

Lower height increase in plants with 100% of branches girdled combined with high number of fruits therein indicates possible competition for assimilation between vegetative and reproducible growth, for while girdling prevents partitioning of assimilates into the root system, it makes more carbohydrates available to the canopy, but does not inhibit competition among the shoot organs. The results corroborate those found by Pereira et al. (2010) when studying fruit fixation in *C. latifólia* girdled plants.

Despite the absence of statistical difference, a trend towards increased foliar concentration of carotenoids in plants with 100% girdling is evident. Accumulation of carbohydrates in the canopy may cause retroinhibition of photosynthesis, hinder CO_2 assimilation and cause oxidative stress (Pereira et al., 2014; Rivas et al., 2007). Under such circumstances, carotenoids take on photoprotective importance, as they remove the reactive species that cause oxidative stress. High correlation between carotenoids and the number of fruits is associated to high accumulation of carbohydrates in the canopy and a consequent higher number of fruits in girdled plants. Although the accumulation of carbohydrates and decrease in leaf concentration of carotenoids is leaf senescence clue in girdled plants (Parrott et al., 2010; Tang et al., 2015), there was no visual symptoms of senescence and carotenoid concentrations remained high and we attribute this phenomenon to the photoprotective role of these pigments.

Regardless of the amount of girdled branches, treated plants presented greater fruit productivity to the detriment of vegetative growth. Plants with 50% of girdled branches, however, presented greater productivity (41% superior to the control) and vegetative growth similar to those of the controls. The 50% branch girdling treatment may possibly have provided more assimilates for the development of fruit, while not fully hindering the transportation of assimilates required to support the absorption of mineral nutrients in the root system. According to Pereira et al. (2010), insufficient mineral nutrients in the canopy are a negative effect of branch girdling in *C. latifólia*.

The high negative correlation between juice volume and number of fruits indicates that greater fruit fixation may reduce juice yield and possibly hamper the product's commercial value, since both the number of fruits and the juice volume depend on and compete for assimilates. The plants with 100% of branches girdled presented higher productivity and lower juice volume per fruit. It suggested that inhibited transportation of assimilates to the root system may have hindered nutrient absorption and played a critical role in the plants' vegetative growth and juice yield. Thus, it is evident that such barrier to the transportation of assimilates from the canopy to the root system may hamper commercial quality of the product.

Plants with 50% of branches girdled presented higher productivity, vigorous vegetative growth, considerable juice yield and quality similar to those of the controls. Notwithstanding, further research is required, with analyses of the vegetative and reproducible growth and nutritional status along several years, for validation and recommendation of the girdling practice.

Conclusions

Plants with 50% of branches girdled presented the highest productivity and greatest juice yield per fruit. Plants with 100% of branches girdled presented high productivity and low juice yield per fruit. Fully blocking the transportation of assimilates from the canopy to the root system has negative impact on the fruit juice volume.

Conflict of Interests

The authors have not declared any conflict of interests.

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