

## Effect of some girdling treatments on fruiting behavior and physio-chemical properties of Washington navel orange trees

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**Abstract:** The effects of girdling branches (GB) and girdling limbs (GL) on flowering, fruit set, fruit yield as well as leaf photosynthetic pigments and endogenous hormones content in 16 year-old Washington navel orange (*Citrus sinensis* L. Osbeck) trees were investigated during two seasons (2012-2013). GB increased number of flowers by 34.22 – 41.26%, fruit set by 103.17 – 113.30% and number of harvested fruits/ branch by 164.44 – 272.25% relative to ungirdled trees. GL increased number of flowers by 19.37 – 23.41%, fruit set by 59.73 – 76.77% and number of harvested fruits/ branch by 62.84 – 148.11% relative to ungirdled trees. However, GB slightly decreased fruit weight (7-19%), fruit size (12-18%) and fruit Total Soluble Solids (TSS)/acid ratio (20-25%) compared to control. In girdling treatments, carbohydrates content was 14-153% and 7-74% more in leaves and stems, respectively. This accumulation of carbohydrates was accompanied by a reduction in total chlorophyll (-38 to -70%) and an increase in carotenoids (+41 to +119%) within the leaves. In young leaves, GB increased Abscisic Acid (ABA) and decreased Gibberellins (GA<sub>3</sub>) and Indole-3-Acetic Acid (IAA) contents, whereas GL increased GA<sub>3</sub> and decreased ABA & IAA concentrations. These results suggested that the accumulation of carbohydrates in girdled branches and limbs enhanced fruit set and fruit yield after girdling.

**Keywords:** Girdling, Navel orange, Endogenous hormones, Fruit retention (%), Pigments, TSS/acid ratio.

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### I. Introduction

Citrus trees are the main fruit crop in Egypt. Washington navel orange is the most popular orange cv. for local consumption and exportation. However, in the last few years the orange fruit trees growing costs increased progressively due to the augmentation of production requirements prices. In addition, the average orange yield per hectare was 23.47 ton compared to 24.68 ton in Brazil and 32.58 ton in United States of America according to the FAO statistics [1]. Therefore, any available practice should be studied to enhance the yield, particularly of Washington navel orange trees in the new reclaimed soils. Girdling was previously used in citriculture to increase flowering and improve fruit set [2-4]. Girdling (the removal of a ring of bark around the branch or trunk) is a safe method on the public health. The primary effect of girdling is the blocking of the downward flow of photoassimilates (carbohydrates), thus provokes the accumulation of these compounds in orange tree above the girdle. The accumulation of carbohydrates in the canopy provides a rich source of energy for flowering, fruit set and development [5].

The effects of girdling have been attributed to the interruption of the downward phloem transport, thereby increasing carbohydrate availability [2-4] and modifying the hormonal balance in the canopy [6]. However, in some cases girdling may induce excessive accumulation of carbohydrates originating a feedback inhibition of photosynthesis by reducing photosynthetic capacity [3] and CO<sub>2</sub> assimilation rate [7]. Other researchers reported that the buildup of carbohydrate reserves increase the size and number of starch granules in chloroplasts causing physical damage to the thylakoid ultrastructure [8, 9], which may explain the symptoms of leaf chlorosis often appear after the treatment [10]. Mechanical damage by girdling elicits several signaling pathways including the increase of endogenous abscisic acid (ABA) [11]. ABA plays a crucial role in the adaptation to several environmental stresses [12]. ABA has been shown to induce the rise of carotene content [11, 13].

The aim of the present study was to investigate the effects of girdling different types of branches on flowering, fruit set percentage, fruit yield and fruit quality as well as leaf photosynthetic pigments, carbohydrate and endogenous hormones contents of Washington navel orange trees.

## **II. Materials and Methods**

### **2.1. Plant Material and Growth Conditions**

The present work was planned to evaluate the effect of some girdling treatments on flowering and fruiting aspects of Washington navel orange trees (*Citrus sinensis* L. Osbeck) during the two successive seasons of 2012 and 2013. The trees were about 16-year-old budded on sour orange rootstock and planted in clay soil at 5 x 5 meters in a private citrus orchard in Minia El-Kamh District, Sharkia Governorate, Egypt. All involved trees were healthy and approximately of the uniform vigour and size, grown under similar agro-climatic conditions. The experimental trees were subjected to the recommended horticultural practices adopted in the commercial citrus orchards in respect of irrigation, fertilization, pruning and pest control.

### **2.2. Girdling Treatments**

The experiment involved three treatments: (1) Control (ungirdled trees), (2) girdling four branches (1-2 cm diameter) at different tree directions and (3) girdling one limb per tree (about 10 cm diameter). Girdling was carried out before anthesis (mid-February) through each season by removing about a 3-4 mm width of the bark around the branch using a girdling knife without injuring the wood.

### **2.3. Flowering and Fruiting Parameters**

Emerged flowers on 4 branches at different tree directions were counted at the balloon stage beginning at the end of March of each season. After fruit set, the remaining fruitlets were counted at the same branches at the end of April (25 – 30 April in the two seasons). Consequently, the fruit set percentage was calculated. The retained fruits were recounted by the end of June and then in December of each season to record number of retained fruits after June drop and number of harvested fruits per branch. In addition, fruit retention percentage was calculated at the harvest date in relation to the number of flowers by the end of December in each season. The retained fruits on each branch were picked and counted and the average number of fruits per branch was calculated.

### **2.4. Fruit Quality parameters**

Fifteen harvested fruits were randomly taken from each replicate (3 replicates) to determine the following fruit characteristics: fruit weight (g) and size (cm<sup>3</sup>), average pulp and peel weights (g), fruit dimensions [height and diameter (cm)], peel thickness (mm), juice volume per fruit (cm<sup>3</sup>).

Navel orange fruits were pressed for 3 min using a Moulinex blinder (Type 716, France) at the maximum speed to extract juice. Prior to analysis and further processing, the juice was centrifuged at 10000 xg (4 °C, for 10 min) to separate the supernatant for the following chemical parameters. Titratable acidity in fruit juice was determined as citric acid by titration against 0.1 N sodium hydroxide solution and the total acidity percentage was calculated [14]. The total soluble solids percentage (TSS %) was determined in fruit juice using a hand refractometer; the TSS/acid ratio was calculated. Vitamin C (ascorbic acid) content as mg per 100 ml juice was determined in fruit juice by titration against 2, 6– dichlorophenol-indophenol dye [14].

### **2.5. Leaf and Stem Chemical Analysis**

In mid-March, samples of new leaves of spring growth cycle (10 g) were taken from non-fruiting branches and placed immediately in methyl alcohol 80 % (v/v). HPLC was used to determine acidic hormones such as IAA, ABA and GAs were determined according to the method described by Shindy and Smith [15].

In September of each season, mature leaf samples were taken randomly from the medium portion of non-fruiting twigs of the spring growth cycle to determine the photosynthetic pigments content. About 0.1 g from each fresh leaf sample was taken for estimating both chlorophyll a & b and carotenoids pigments according to the method described by Wettstein [16].

For estimating leaf and stem carbohydrates contents, samples were frozen immediately in liquid N<sub>2</sub>, lyophilized and stored as powders at –28°C. Samples (100 mg) of dry matter were extracted with 1.0 ml 80% (v/v) ethanol. The extracts were incubated at 85°C for 5 min and centrifuged at 15,000 xg for 10 min. The supernatant was removed, the pellet was re-extracted twice as above, and the combined supernatants were evaporated *in vacuo* at 45°C [17].

### **2.6. Statistical Analysis**

The experiment was arranged in a complete randomized block design with three replicates, each consisted of three trees. The obtained data were statistically analyzed according to Snedecor and Cochran [18] using SAS software [19]. The individual comparisons between the obtained values were carried out using LSD at 0.05 level to compare the means.

### **III. Results and Discussion**

#### **3.1. Effect of Girdling Treatments on Floral and Fruiting Characteristics of Washington Navel Orange Trees**

Data in Table (1) show that number of flowers per branch of Washington navel orange trees was significantly affected by the tested girdling treatments in the two seasons. The highest numbers of flowers per branch (329.44 & 330.56 flowers) were recorded for girdled branches against 293.00 & 288.78 flowers for girdled limbs in the first and second seasons, respectively, without significant differences between them in the two seasons. The Control (ungirdled) trees produced significantly lower numbers of flowers per branch in the two tested seasons (245.45 & 234.00 flowers, respectively). These results are in harmony with those reported by Banchongsiri [20] on lime and Agusti et al. [21] on sweet orange and mandarin. They all reported that branch and stem girdling significantly promoted flowering.

Girdling branches and limbs significantly affected the initial number of fruitlets per branch and fruit set percentage in the two seasons. Data in Table (1) show that the highest numbers of fruitlets per branch (115.89 & 132.33 in the first and second seasons, respectively) were recorded for girdled branches, followed by girdled limbs (91.11 & 109.67). Meanwhile, control treatment exhibited the lowest numbers of fruitlets (57.04 & 62.04 for the control) in the first and second seasons, respectively.

Girdling treatments yielded higher initial fruit set percentages (35.18 & 40.04 % for girdling branches and 31.10 & 37.98 % for girdling limbs) in the first and second seasons, respectively without significant differences between them. Whereas, the Control treatment gained the lowest initial fruit set percentages (23.24 and 26.53 % in the two seasons, respectively). These results are in line with those of Fishler et al. [22] on grapefruit, Mataa et al. [23] on Ponkan mandarin, Mustafa and Saleh [24] on Balady mandarin, who found that girdling increased fruit set percentage in all cultivars. Also, Rivas et al. [2, 25] on Clemenules mandarin found that girdling can improve and enhance fruit set percentage by early modification of carbohydrates and GAs/ABA relationship regardless of shoot type.

Girdling treatments had a significant effect on number of retained fruits and fruit retention percentage in both seasons. Data in Table (1) show that number of retained fruits was highest with girdling branches treatment (24.00 and 29.67) against only 8.33 and 8.77 fruitlet/ branch, for the Control in the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively. Girdling limbs gave in-between values (15.55 and 20.22 fruitlet/ branch). The fruit retention percentage, in relation to initial fruit set, indicated a similar trend being highest with girdling branches (20.90 & 22.43 %), and followed by girdling limbs (17.07 & 18.44 %, in the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively). The least fruit retention percentages were recorded by the Control (14.60 & 18.44 %). Similar conclusion has been reported by Fishler et al. [22], Agusti et al. [21, 26], Wright [27], Mustafa and Saleh [24], Rivas et al. [2, 3]. They all found increased fruit retention percentage and yield of most of citrus species by girdling. On the other hand, Rivas et al. [25] on Clemenules mandarin found that fruit retention percentage was not significantly affected by girdling.

Data at harvest followed the same trend, since the highest numbers of the harvested fruits (18.22 & 22.67/ branch in the two seasons) and the highest final fruit retention percentage in relation to number of flowers (5.54 & 6.86 %) came from girdling branches. The least values, i.e. 6.89 & 6.09 fruits and 2.82 & 2.60 % came from the Control. Girdling limbs gave in-between values, i.e. 18.22 & 22.67 fruits per branch and 3.83 & 5.23 % for the final fruit retention percentage. Girdling branches increased number of the harvested fruits/ branch by 264.45 & 372.25 % in relation to Control in the 1<sup>st</sup> & 2<sup>nd</sup> seasons, respectively. This would be a great advance in Washington navel orange production if the leaf surface area can provide enough assimilates. A proceeding study must determine the necessary fertilization regime.

#### **3.2. Effect of Girdling Treatments on Physical Properties of Washington Navel Orange Fruits**

Table (2) illustrates the effect of the tested girdling treatments on fruit weight and size, peel and pulp weights, peel thickness, fruit length and diameter and juice volume per fruit. However, the data cleared that in both seasons the largest and heaviest fruits as well as the heaviest pulps, peels and juice volume per fruit were obtained by Control (ungirdled) branches and girdling limbs treatments without significant differences between them in most cases. On the other hand, girdling branches, which gained the uppermost numbers of fruits, gave significantly lower values in all these criteria in both seasons. For example, the average fruit and pulp weights with girdling branches were 183.64 and 129.80 gm in the first season and 206.66 and 139.90 gm in the second season, respectively. The corresponding values for Control (ungirdled) trees were 225.50 and 163.33 gm in the first season and 221.96 and 156.47 gm in the second one. Apparently such results might be due to a reduction in the leaf/fruit ratio due to the increments in fruit numbers by girdling [28]. Such reductions in fruit weights points out to a necessary correction in the fertilization program to accelerate vegetative growth and consequently promote the leaf/fruit ratio.

These results are in line with those reported by Banchongsiri [20] on lime, who found that stem girdling reduced fruit weight. On the contrary, Dawood et al. [29] and Mostafa and Saleh [24] on Balady mandarin found that girdling before blossoming increased fruit weight. Also, Shamel and Pomeroy [30], Ghayur and Khan [31]

and Agusti et al. [26] obtained smaller fruits of Washington navel orange and mandarin by girdling. Meanwhile, Krezdorn [32] and Krezdorn and Wiltbank [33] on 'Orlando' tangelo reported no change in fruit size by girdling. Cohen [28] also suggested that the number of leaves from which a fruit can draw carbohydrates affects fruit size. Girdling too early will lead to too many fruits per leaf, thus reducing fruit size. Girdling too late will lead to a smaller increase in fruit size. Khandaker et al. [34] on wax jambu (*Syzygium samarangense*) found that girdling branches produced the highest fruit length and diameter to about 6.9 and 4.8 cm. On the other hand, Banchongsiri [20] found that stem girdling did not affect lime fruit diameter and length. Mustafa and Saleh [24] reported that the juice weight per fruit was significantly decreased with girdling treatments comparing with the Control trees, which recorded the highest juice weight per fruit.

### **3.3. Effect of Some Girdling Treatments on Some Chemical Constituents of Washington Navel Orange Juice**

In both seasons, it is clear that girdling branches significantly decreases TSS % and the TSS/ acid ratio in the fruit juice, while, promoted juice acidity particularly in the second season, (Table 3). These results pointed out those girdling branches might have delayed fruit maturities which have relation with the increasing number of fruits by such a treatment, and consequently the reduction in leaf/ fruit ratio. Also, reduced SS/fruit due to fruit to leaf ratios and smaller leaves than delayed maturity and may be due to the excessive accumulation of carbohydrates which the opposite effect on the content of sugars. The data also reveal a significant increase in juice Vit. C content by girdling limbs compared with un-girdling and girdling branches. This was statistically significant in the first season only.

Literature reports on the effects of girdling on some chemical constituents of the juice are a contradiction. Both Dawood et al. [29] and Rivas et al. [2] on mandarin cvs., found that girdling treatments improved and increased the soluble sugars content (SSC) in fruit juice. Mustafa and Saleh [24] reported that girdling alone and plus potassium spray at pre-bloom enhanced soluble solids content and SSC/acid ratio and had no effect on acidity. Roussos and Anastassios [35] reported that sucrose, glucose and fructose concentrations were highest in fruits from girdled scaffold of mandarin trees. Moreover, for total soluble solids per fruit and total fruit to account for change in number of fruit, Branch girdling will keep more leaf photosynthates in fruiting area and limb girdles as another factor. In contrast, Banchongsiri [20] found that stem girdling reduced total soluble solids (TSS) in lime fruits. Moreover, Dawood et al. [29] and Mustafa and Saleh [24] found that girdling treatments did not affect ascorbic acid content (vitamin C) in fruit juice of Balady mandarin.

### **3.4. Effect of Girdling Treatments on Gibberellins (GA), Indole-3-Acetic Acid (IAA) and Abscisic Acid (ABA) Contents in Young Leaves of Washington Navel Orange Trees**

As shown in Figure (1), young leaves of girdled branches, which revealed the highest flowering and fruiting activities, indicated the lowermost contents of GA<sub>s</sub> (16.14 & 18.20 ppm) and IAA (0.59 & 0.46 ppm) in the 1<sup>st</sup> & 2<sup>nd</sup> seasons, respectively. On the other hand, girdling limbs recorded the highest GA<sub>3</sub> content in both seasons (60.48 & 68.21 ppm, respectively), while leaves of the Control (ungirdled) trees indicated intermediate contents (22.31 & 25.16 ppm, respectively). The highest level of the endogenous IAA content of the young leaves was recorded for Control (ungirdled) trees being 1.90 & 1.49 ppm in 2012 and 2013 seasons, respectively. Girdling limbs came in the second rank (1.57 & 1.24 ppm, respectively). The lowermost IAA concentrations were obtained by girdling branches (0.59 & 0.46 ppm, respectively). The data showed also that girdling branches gained the highest ABA content in young leaves, descending followed by the Control, while girdling limbs occupied the last position in this respect. GA<sub>1/3</sub> content was higher in the leaves of bearing shoots than in those of the vegetative ones [36]. Rivas et al. [37] girdled trunk of mandarin seedling in spring and found that girdling increased abscisic acid in young leaves and decreased it in mature leaves. Mahouachi et al. [38] found that girdling increased GA concentration when performed on branches of Satsuma mandarin at anthesis.

### **3.5. Effect of Girdling Treatments on Total Carbohydrates, Chlorophyll A, B And Carotenoids Pigments Contents in Washington Navel Orange Leaves**

Figure 2 show chlorophyll a, b and total chlorophyll (a+b) contents in the Washington navel orange leaves as affected by the tested girdling treatments. The data clarified that girdling branches or limbs significantly affected the chlorophyll a and total chlorophyll contents of Washington navel leaves in both seasons and chlorophyll b in the first one. However, it seems that girdling, generally, tended to decrease chlorophyll values in both seasons compared to the Control.

In addition, girdling branches or limbs significantly promoted carotenoids content of Washington navel leaves as compared to the Control (data not shown). The obtained results were in general agreement with Vemmos et al. [39] reported that the concentrations of Chl. a, Chl. b, total chlorophyll (a+b), as well as the ratio of Chl. a/b, were all decreased with girdling pistachio shoots. In contrast, Mustafa and Saleh [24] on Balady mandarin who found that girdling plus potassium sprays had a positive effect on total chlorophyll and chlorophyll (a) content in the leaves, whereas reduced chlorophyll (b) content. On the other hand, Rivas et al. [3] reported that girdling trunk

few weeks before flowering of mandarin trees failed to induce any differences in total chlorophyll concentrations between Control and girdled trees.

Data in Figure 3 clearly show that girdling branches increased total carbohydrates percentages in both leaves and stem compared to the Control (ungirdled) or girdling limbs. The values were 11.67 and 25.33 % for leaves in 2012 & 2013, respectively against 9.00 & 10.00 %, respectively for the Control and 10.67 & 19.00 % for girdling limbs. Girdling branches and girdling limbs gave statistically equal values in 2012.

Regarding carbohydrates contents in stem, values showed a trend similar to that discussed above for the leaves. The highest values (9.00 & 13.33 % in 2012 & 2013, respectively) were for girdling branches. Girdled limbs had intermediate values (8.00 & 10.67 %, respectively). The lowest values were for the Control (7.33 & 7.67 %, respectively). Differences between the three treatments were statistically significant in the two seasons.

In this respect, assimilates may accumulate directly above the girdle, but generally increased levels of carbohydrates can be found throughout the canopy accordingly, its effects are brought about by the increased availability of carbohydrates [5, 40]. Roper and Williams [41] demonstrated that foliar carbohydrates were higher in girdled vines four weeks after the girdling treatment and concomitantly root carbohydrate concentrations were lower in the untreated Control. This accumulation ranged by 10 – 30 % of the content in the leaves of the ungirdled Control. Girdling the scaffold branches caused the accumulation of carbohydrates in the leaves of the off trees [42]. Vemmos et al. [39] found that girdling increased carbohydrates, particularly starch in pistachio shoots.

#### IV. Conclusions

Different types of girdling particularly branches and limbs can increase yield, number of flowers, set fruitlets and retained fruits/ branch of Washington navel orange. The number of harvested fruits from girdled branches was two to three folds higher than those of the Control. On basis of the obtained results it might be suggested to use branches girdling of Washington navel orange trees, to obtain clear increments in fruit set and fruit retention percentages. In addition, an intensified fertilization program must be accompanied to promote leaf/ fruit ratio and the fruit physical and chemical properties

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#### Conflict of Interests

The authors have not declared any conflict of interests.

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**Table (1):** Effect of some girdling treatments on floral and fruiting characteristics of Washington navel orange trees (2012 and 2013 seasons)

Treatments	Number of flowers/ branch	initial number of fruitlets/ branch	Initial fruit set <sup>a</sup> (%)	Number of retained fruits/ branch after June drop	Fruit retention after Junedrop <sup>b</sup> (%)	Number of harvested fruits/ branch		Final fruit retention (%) at harvest
						value	± % *	
<b>First season (2012)</b>								
Control	245.45	57.04	23.24	8.33	14.60	6.89	-	2.82
Girdling branches	329.44	115.89	35.18	24.22	20.90	18.22	164.45	5.54
Girdling limbs	293.00	91.11	31.10	15.55	17.07	11.22	62.85	3.83
LSD at 0.05	44.28	22.60	8.86	2.15	4.14	1.85	-	0.65
<b>Second season (2013)</b>								
Control	234.00	62.04	26.53	8.77	14.14	6.09	-	2.60
Girdling branches	330.56	132.33	40.04	29.67	22.43	22.67	272.25	6.86
Girdling limbs	288.78	109.67	37.98	20.22	18.44	15.11	148.12	5.23
LSD at 0.05	47.54	15.40	9.14	2.41	3.53	1.68	-	1.37

a In relation to number of flowers

b In relation to initial number of fruitlets

c In relation to number of flowers

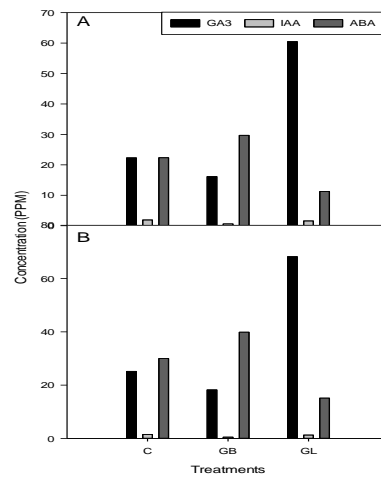
\* ± % in number of fruits/ branch in relation to Control

**Table (2):** Effect of some girdling treatments on physical properties of Washington navel orange fruits (2012 and 2013 seasons)

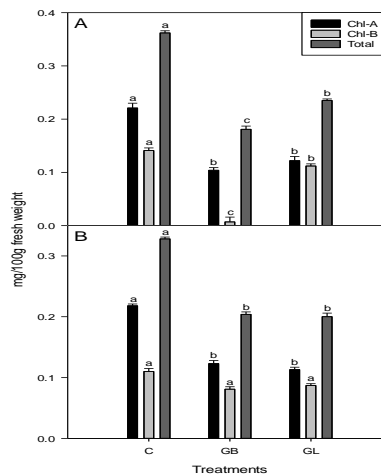
Treatments	Fruit weight (g)	Fruit size (cm <sup>3</sup> )	Pulp weight (g)	Peel weight (g)	Peel thickness (mm)	Juice volume/ fruit (cm <sup>3</sup> )	Fruit length (cm)	Fruit diameter (cm)
<b>First season (2012)</b>								
Control	225.50	241.96	163.33	59.80	4.27	112.31	7.93	7.54
Girdling branches	183.64	199.04	129.80	42.20	3.35	92.00	7.08	7.12
Girdling limbs	223.24	244.40	169.13	57.73	3.92	116.29	8.01	7.61
LSD at 0.05	31.63	34.29	20.18	10.06	0.87	17.95	0.38	0.41
<b>Second season (2013)</b>								
Control	221.96	240.57	156.47	62.80	4.65	107.58	7.96	7.48
Girdling branches	206.66	210.66	139.90	42.93	3.51	96.31	7.39	7.17
Girdling limbs	246.66	267.34	183.53	63.53	4.43	126.19	8.17	7.67
LSD at 0.05	35.07	29.11	16.42	11.84	0.35	11.43	0.32	0.48

**Table (3):** Effect of some girdling treatments on some chemical constituents of Washington navel orange juice (2012 and 2013 seasons)

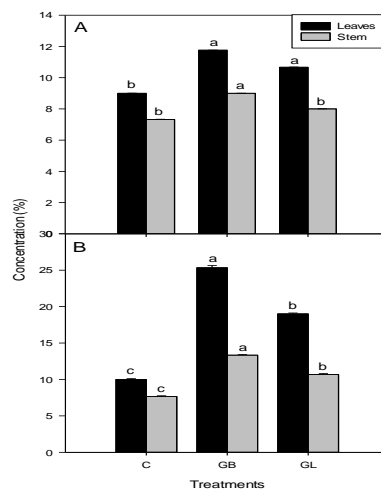
Treatments	TSS (%)	Acidity (%)	TSS/ acid ratio	Vitamin C content (mg/ 100 ml juice)
<b>First season (2012)</b>				
Control	14.53	0.83	17.52	59.16
Girdling branches	13.20	0.94	14.06	54.15
Girdling limbs	15.13	0.79	19.37	71.19
LSD at 0.05	0.71	NS	5.13	8.77
<b>Second season (2013)</b>				
Control	15.27	0.79	19.46	63.67
Girdling branches	12.53	0.85	14.69	60.16
Girdling limbs	15.40	0.77	20.15	66.20
LSD at 0.05	0.61	0.05	1.53	NS



**Figure 1.** Effect of some girdling treatments on gibberellins (GA<sub>3</sub>), indole-3-acetic acid (IAA) and abscisic acid (ABA) contents in young leaves of Washington navel orange trees [2012 (A) and 2013 (B) seasons]. C: Control; GB: girdling branches; GL: Girdling limbs.



**Figure 2.** Effect of some girdling treatments on total photosynthetic pigments contents in Washington navel orange leaves [2012 (A) and 2013 (B) seasons], C: Control; GB: girdling branches; GL: Girdling limbs.



**Figure 3.** Effect of some girdling treatments on total carbohydrate contents in Washington navel orange leaves and stem [2012 (A) and 2013 (B) seasons], C: Control; GB: girdling branches; GL: Girdling limbs.