# Root confinement and irrigation frequency affect growth of 'Rough lemon' (*Citrus limon*) seedlings

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### Root confinement and irrigation frequency affect growth of 'Rough lemon' (*Citrus limon*) seedlings.

**Abstract** — **Introduction**. Root restriction occurs when seedlings are grown in increasingly small containers and it reduces plant growth in different ways. Therefore, the objectives of our study were to investigate the effects of varying container sizes and irrigation frequency on growth parameters of 'Rough lemon' (C. limon) plants Maseno, Kenya. Materials and methods. The treatments tested the effect of three container volumes (1.7 L, 2.7 L and 4.5 L) and three irrigation frequencies [every day  $(W_1)$ , every two days  $(W_2)$  and every 3 days  $(W_3)$ ]. The design of the experiment was completely randomized with three replications. Growth parameters studied were plant height, canopy height, shoot and root weight, number of leaves and shoot to root ratio. Results and discussion. Increasing container volumes generally increased the height of plants, height of canopy, weight of whole plants and weight of roots, but it did not affect number of leaves, stem diameter or shoot to root ratio. Irrigation frequency increased number of leaves, height of plants, height of canopy, stem diameter and weight of shoots and roots, but this factor had no effect on weight of whole plants. These findings are explained by reduced root restriction in the largest container volumes. **Conclusion**. Increasing container volumes from 1.7 L to 4.5 L and irrigation frequency from every 3 days to every day increases plant growth as expressed in the various plant growth parameters.

Nigeria / *Citrus limon* / plant propagation / plant nurseries / planting stock / growth / container planting / irrigation

Le confinement des racines et la fréquence d'irrigation affectent la croissance de jeunes plants de 'Rough lemon' (*Citrus limon*).

**Résumé** — **Introduction**. Un confinement des racines a lieu lorsque de jeunes plants se développent dans des containers de petites tailles et cela réduit la croissance des plantules de différentes manières. Les objectifs de notre étude ont donc été d'étudier les effets de la taille des containers et de la fréquence d'irrigation sur des paramètres de croissance de plants de 'Rough lemon' (C. limon) observés en pépinière à Maseno, Kenya. Matériel et méthodes. Les traitements ont testé l'effet de trois volumes de container (1,7 L, 2,7 L et 4,5 L) et de trois fréquences d'irrigation [journalière (W1), tous les 2 jours (W2) et tous les 3 jours (W3)]. Le dispositif expérimental, présentant trois répétitions, a été complètement randomisé. Les paramètres de croissance étudiés ont été la hauteur de la plante, la hauteur de la frondaison, le poids de la tige et des racines, le nombre de feuilles et le rapport [tige / racines]. Résultats et discussion. L'augmentation du volume des containers a généralement augmenté la taille des plants et celle de la frondaison, le poids du plant entier et celui des racines, mais il n'a pas affecté le nombre de feuilles, le diamètre de tige et le rapport [tige / racines]. La fréquence d'irrigation a augmenté le nombre de feuilles, la taille des plants et celle de la frondaison, le diamètre de tige et le poids des tiges et des racines, mais ce facteur n'a eu aucun effet sur le poids des plants entiers. Ces résultats sont expliqués par un meilleur développement des racines dans les containers de plus grands volumes. Conclusion. En augmentant le volume des containers de 1,7 L à 4,5 L et la fréquence d'irrigation d'un arrosage tous les 3 jours à un arrosage journalier, la croissance de plantes, exprimée par divers paramètres de croissance, s'est révélée améliorée.

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RESUMEN ESPAÑOL, p. 202

Nigéria / *Citrus limon* / multiplication des plantes / pépinière / plant / croissance / plantation en container / irrigation

#### Table I.

Effect of irrigation frequency and root restriction on number of leaves and height of plants of 'Rough Lemon' seedlings grown in Maseno, Kenya.

| Container volume (L)  | Irrigation frequency <sup>1</sup> | Number of leaves | Height of plants (cm) |
|---|-----------------------------------|------------------|-----------------------|
| 1.7 (V <sub>1</sub> )   | W <sub>1</sub>                    | 23.7             | 28.7                  |
|   | W <sub>2</sub>                    | 28.3             | 61.3                  |
|   | W <sub>3</sub>                    | 15.3             | 32.0                  |
| 2.7 (V <sub>2</sub> )   | W <sub>1</sub>                    | 44.3             | 67.3                  |
|   | W <sub>2</sub>                    | 72.7             | 60.1                  |
|   | W <sub>3</sub>                    | 22.7             | 50.0                  |
| 4.5 (V <sub>3</sub> )   | W <sub>1</sub>                    | 63.0             | 115.3                 |
|   | W <sub>2</sub>                    | 62.3             | 99.7                  |
|   | W <sub>3</sub>                    | 30.7             | 69.7                  |
| Statistical parameter   |                                   | Number of leaves | Height of plants (cm) |
| LSD <sup>2</sup> between irrigation frequency means                                     |                                   | 18.8             | 19.3                  |
| _SD <sup>2</sup> between container sizes  |                                   | 15.7             | 16.4                  |
| LSD <sup>2</sup> [container size × irrigation frequency]                                |                                   | 9.2              | 17.5                  |
| Significance of <i>F</i> tests <sup>3</sup> for container sizes                         |                                   | Significant      | Significant           |
| Significance of <i>F</i> tests <sup>3</sup> for irrigation frequency                    |                                   | Significant      | Significant           |
| Significance of <i>F</i> tests <sup>3</sup><br>for [container sizes × irrigation freque | maul                              | Not significant  | Not significant       |

<sup>1</sup> W<sub>1</sub>: every day, W<sub>2</sub>: every 2 days, W<sub>3</sub>: every 3 days.

<sup>2</sup> LSD at  $P \le 0.05$ .

<sup>3</sup> F test at  $P \leq 0.05$ .

### 1. Introduction

Root restriction occurs when fruit tree seedlings are grown in increasingly small containers. There are two options for commercial nursery producers; namely, to plant young seedlings directly into market size containers or transplant them into smaller containers and later transfer them into market size containers (upcanning). While upcanning is more labor-intensive, it requires less shape and it shades the container more quickly, thereby reducing temperatures in the growth medium and crop failure [1].

Increasing container size increases canopy growth [2–6] in pears (*Pyrus calleryana*), pecan (*Carya illinoensis*), Japanese euonymus (*Euonymus japonica* Thumb) and other ornamental species, respectively. Conversely, growing seedlings in small containers causes root restriction which, in turn, reduces canopy growth [7-10], plant growth expressed as shoot length, fresh weight, dry weight accumulation and leaf area [11]. Small containers allow less expansion and caliper development of plants, and reduce the number of primary shoots; total combined length of secondary shoots and total length of all shoots [12] reduce CO2 assimilation and leaf conductance [13], reduce leaf nutrient levels except N [13], and reduce the dry weights of roots, stems, leaves and fruit [14]. Although root restriction reduces dry matter production and K concentration in plant organs, it does not cause nutrient deficiency [9, 15]. However, Bar-Tal et al. [14] have reported that root restriction reduces both dry matter production and K concentration in plant organs including the roots. Other reports have indicated that root restriction retards plant growth by reducing hormone synthesis and metabolism in the root system [8, 16].

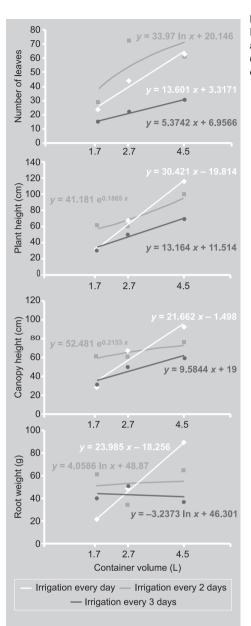
Rootstocks used in Kenya are 'Rough lemon' (*Citrus limon*). There has been no research conducted previously on the growth responses of Rough lemon to varying container sizes or root confinement and irrigation frequency. These are critical issues affecting fruit tree nursery production in Kenya. Therefore, the objectives of our study were to investigate the effects of varying container sizes and irrigation frequency on the growth parameters of Rough lemon rootstocks in Maseno, Kenya. The hypothesis of this study was that root confinement and irrigation frequency affect the growth of *C. limon*.

#### 2. Materials and methods

#### 2.1. Location of research site

The study was conducted at the Maseno University, Kenva. The nurseries of the department of horticulture are situated at an altitude of 1515 m above sea level and at long. 34° E and 36° E and lat. 0°. The soils comprise a complex of somewhat excessively drained, shallow, stony and rocky soils of varying color, consistency and texture of dystric regosols with ferralic cambisols, lithic phase and rock outcrops. The soil is acidic with high extractable Ca and K. Soil organic carbon content is 1.8% and phosphorus content is 4.5 mg·kg<sup>-1</sup>, respectively. The pH of the soil ranges between 4.5 and 5.4 [17]. The soil has a water holding capacity of 40%. The area receives a fairly well distributed annual rainfall of 1853 mm.

On March 12, 1998, Rough lemon fruits were harvested from citrus trees managed according to the standard practices of weed control, irrigation, fertilization and pest and disease control [18]; then, they were transported to Maseno university laboratories, 20 km away and stored in the refrigerator for 2 days at a temperature of 5 °C. The fruits were then washed and graded for uniform-



ity of mass and freedom from blemishes and subsequently cut in half and seeds extracted from them in warm water at a temperature of 70 °C [19]. The seeds were then dried briefly in trays in the laboratory for 2 days in readiness for planting in the field. Figure 1.

Effect of irrigation frequency and container volume on some *Citrus limon* seedling characteristics.

#### Table II.

Effect of irrigation frequency and container size on the height of canopy and stem diameter of 'Rough Lemon' seedlings grown in Maseno, Kenya.

| Container volume (L)   | Irrigation frequency <sup>1</sup> | Height of canopy (cm) | Stem diameter<br>(cm) |
|--|-----------------------------------|-----------------------|-----------------------|
| 1.7 (V <sub>1</sub> )  | W <sub>1</sub>                    | 28.7                  | 2.3                   |
|  | W <sub>2</sub>                    | 61.3                  | 3.5                   |
|  | W <sub>3</sub>                    | 32.0                  | 2.7                   |
| 2.7 (V <sub>2</sub> )  | W <sub>1</sub>                    | 67.3                  | 3.3                   |
|  | W <sub>2</sub>                    | 60.1                  | 2.8                   |
|  | W <sub>3</sub>                    | 50.0                  | 3.3                   |
| 4.5 (V <sub>3</sub> )  | W <sub>1</sub>                    | 92.3                  | 3.7                   |
|  | W <sub>2</sub>                    | 75.3                  | 3.5                   |
|  | W <sub>3</sub>                    | 60.3                  | 3.3                   |
| Statistical parameter  |                                   | Height of canopy (cm) | Stem diameter<br>(cm) |
| LSD <sup>2</sup> between irrigation frequency means                                    |                                   | 18.7                  | 3.7                   |
| LSD <sup>2</sup> between container sizes   |                                   | 17.0                  | 1.0                   |
| LSD <sup>2</sup> [container size × irrigation frequency]                               |                                   | 18.9                  | 1.1                   |
| Significance of $F$ tests <sup>3</sup> for container sizes                             |                                   | Significant           | Significant           |
| Significance of $F$ tests <sup>3</sup> for irrigation frequency                        |                                   | Significant           | Not significant       |
| Significance of $F$ tests <sup>3</sup> for<br>[container sizes × irrigation frequency] |                                   | Not significant       | Not significant       |

<sup>1</sup> W<sub>1</sub>: every day, W<sub>2</sub>: every 2 days, W<sub>3</sub>: every 3 days.

<sup>2</sup> LSD at  $P \le 0.05$ .

<sup>3</sup> *F* test at  $P \le 0.05$ .

### 2.2. Land preparation, fertilization, planting and subsequent care

Nursery beds measuring  $1.5 \text{ m} \times 15 \text{ m}$  were well prepared using hoes, rakes, machetes and trowels and mixed thoroughly with 8 kg of farmyard manure and 100 kg·ha<sup>-1</sup> of diammonium phosphate (Kenya Farmers Assoc., Nakuru, Kenya) NPK fertilizer (18-46-0). The seeds were then planted in the nursery beds on March 15, 1999, at a spacing of 40 cm between the rows and 15 cm within the rows. A layer of grass mulch was spread between the holes. The beds were watered twice daily at 08:00 h and 17:00 h to saturation. For pest control, Aldrin at 40% exchangeable cations (EC) was applied at a rate of 5 g·kg<sup>-1</sup> of seed for the control of cut worms, while Dimethoate (dimethyl-5 (N-methyl carbomethyl) phosphorothiolo thionate) at 40% EC was sprayed at a rate of 1 L·500 L<sup>-1</sup> of water·ha<sup>-1</sup> at 2-week intervals to control other insect pests. Diseases were controlled by Benomyl (methyl N (1-butyl carbomyl-2-benzimidazole) carbamate) at a rate of 20 g·L<sup>-1</sup> of water. Irrigation was carried out by watering cans with a 16-mm interval diameter hose and breaker nozzle. Weeds were controlled by hand pulling.

# 2.3. Transplanting of seedlings, treatments and experimental design

Uniform seedlings were transplanted on July 15, 1999, into three sizes of plastic pots according to the treatments to be applied, which reflected different degrees of root confinement. A media of [1 vol. sand:1 vol. soil] was used. Diammonium phosphate 18-46-0) NPK fertilizer (Kenya Farmers Assoc., Nakuru, Kenya) was added to the pots at a rate of 24 g per pot every month. The pots were spaced at a distance of  $1 \text{ m} \times 1 \text{ m}$  in a 30% shade structure made of timber and covered with grass on the top. The area of the shade structure was approximately  $200 \text{ m}^2$ . Average daily temperatures were 26 °C and relative humidity was 58%. Pest and disease control was carried out as described above for nursery seedlings, while weed control was done manually using hoes and machetes.

## 2.4. Experimental design and treatment

The treatments comprised three container sizes, namely 1.7 L (V<sub>1</sub>), 2.7 L (V<sub>2</sub>) and 4.5 L (V<sub>3</sub>), representing three degrees of root confinement (high, medium and low, respectively) and three irrigation frequencies: irrigating to saturation every day and twice a day (W<sub>1</sub>), irrigating to saturation every two days and twice a day (W<sub>2</sub>) and irrigating every 3 days and twice a day (W<sub>3</sub>). The statistical design used was completely randomized with four replications. There were four plants per replication.

#### 2.5. Sampling and statistical analysis

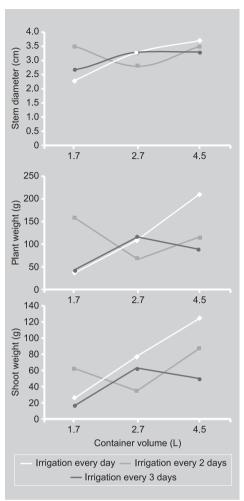
Sampling was conducted destructively. The plants were uprooted on March 15, 2000. They were washed free of soil, dried at 70 °C for 48 h in an oven and, then, their leaves, stems, roots and shoots were weighed. Plant height was measured from the lowest point of the canopy to the highest. Stem diameter was measured by a Veneer caliper at 5 cm from the level of media in the pots. The root to shoot ratio was determined by dividing their dry weights. Four plants per replication per treatment were sampled. A statistical analysis package (SAS Institute [20]) was used for analysis of variance and separation of means determined by Least Significant Differences (LSD) at ( $P \le 0.05$ ). Regression analysis was also done.

#### 3. Results

Number of leaves was significantly increased by container size and irrigation frequency, but the interaction [container size × irrigation frequency] was not significant (*table I*, *figure 1*). The same goes for the height of plants. Irrigation every day ( $W_1$ ) or every two days ( $W_2$ ) produced taller plants than irrigation every 3 days ( $W_3$ ) (*table I*, *figure 1*).

Height of canopy was significantly increased by container size and irrigation frequency (*table II, figure 1*). However, the [container size × irrigation frequency] interaction was insignificant. As the container size increased from 1.7 L to 4.5 L, plants of  $W_1$  and  $W_2$  treatments had much taller canopies than  $W_3$ . Stem diameter was affected by the container size, but not by irrigation frequency (*table II*). The interaction [container size × irrigation frequency] was not significant (*figure 2*).

Weight of whole plants was increased by container size but not by irrigation frequency (*table III*). However, the [container size × irrigation frequency] interaction was significant (*table III, figure 2*). Although there were variable results in the weight of whole plants at the container size of 2.7 L, there were definite trends for the other container sizes, *i.e.*, increasing weights of whole plants



#### Figure 2.

Interaction between irrigation frequency and container volume on some *Citrus limon* seedling characteristics.

as irrigation frequency increased (*table III*, *figure 2*).

Irrigation frequency significantly increased the weight of shoots, which, in contrast, was not affected by the container size. However, the interaction between these factors was significant (*table IV*, *figure 2*). Only irrigation frequency increased the weight of roots and neither container size nor [irrigation frequency x container size] interaction was significant (*figure 1*). The shoot to root ratio was affected neither by irrigation frequency nor by container size (*table IV*). The interaction between the two factors was also insignificant.

#### Table III.

Effect of irrigation frequency and container size on the weight of whole plants and weight of roots of 'Rough Lemon' seedlings grown in Maseno, Kenya.

| Container volume (L)   | Irrigation frequency <sup>1</sup> | Weight of whole<br>plants (g) | Weight of roots (g)    |
|--|-----------------------------------|-------------------------------|------------------------|
| 1.7 (V <sub>1</sub> )  | W <sub>1</sub>                    | 36.8                          | 21.3                   |
|  | W <sub>2</sub>                    | 157.6                         | 61.1                   |
|  | W <sub>3</sub>                    | 43.4                          | 39.8                   |
| 2.7 (V <sub>2</sub> )  | W <sub>1</sub>                    | 109.7                         | 48.4                   |
|  | W <sub>2</sub>                    | 68.7                          | 33.7                   |
|  | W <sub>3</sub>                    | 115.6                         | 52.2                   |
| 4.5 (V <sub>3</sub> )  | W <sub>1</sub>                    | 210.8                         | 89.0                   |
|  | W <sub>2</sub>                    | 117.5                         | 64.1                   |
|  | W <sub>3</sub>                    | 88.4                          | 37.1                   |
| Statistical parameter  |                                   | Weight of whole plants (g)    | Weight of roots<br>(g) |
| LSD <sup>2</sup> between irrigation frequency means                                    |                                   | 153.7                         | 38.0                   |
| LSD <sup>2</sup> between container sizes   |                                   | 37.8                          | 26.7                   |
| LSD <sup>2</sup> [container size × irrigation frequency]                               |                                   | 30.2                          | 35.2                   |
| Significance of <i>F</i> tests <sup>3</sup> for container sizes                        |                                   | Significant                   | Not significant        |
| Significance of $F$ tests <sup>3</sup> for irrigation frequency                        |                                   | Not significant               | Significant            |
| Significance of $F$ tests <sup>3</sup> for<br>[container sizes × irrigation frequency] |                                   | Significant                   | Not significant        |

<sup>1</sup> W<sub>1</sub>: every day, W<sub>2</sub>: every 2 days, W<sub>3</sub>: every 3 days.

<sup>2</sup> LSD at  $P \le 0.05$ .

<sup>3</sup> *F* test at  $P \le 0.05$ .

#### 4. Discussion

In our study, increasing container sizes or volumes generally increased the growth parameters of roots and shoots: number of leaves, height of plants, height of canopy, stem diameter, weight of whole plants and weight of roots. This was possibly due to the fact that the larger containers caused less root restriction, which resulted in increased growth rates [8, 9, 11, 15, 16].

It has been reported that reduced root restriction increases soil volume, nutrient uptake, hormone synthesis and metabolism in the root system [8, 13, 16]. These reasons could be advanced to explain the results of our study, but a follow-up study should be carried out to measure the above mentioned parameters. Under the conditions experienced, there was increased development of primary shoots and the total length of shoots increased [12], thereby producing taller plants and canopies.

Increasing irrigation frequency from irrigation every 3 days  $(W_3)$  to irrigation every day  $(W_1)$  increased C. *limon* seedling growth parameters. This is due to the fact that water is needed for the initial plant growth processes, such as cell division and cell enlargement, and for the subsequent metabolic processes, which are needed for the synthesis of the various components of the plant systems. Water is also a medium in which the nutrients needed for growth are found. In most cases of our study, irrigation every day  $(W_1)$  or every 2 days  $(W_2)$  had similar results in the growth parameters, implying that watering every 2 days  $(W_2)$  is sufficient for plant growth.

### 5. Conclusions

• Increasing container size from 1.7 L to 4.5 L increases shoot and root growth for number of leaves, stem diameter, weight of whole plants, height of canopy and height of plants, but not for weight of roots and shoot and shoot to root ratio.

• Both irrigating every day and irrigating every 2 days generally increase shoot and root growth for number of leaves, height of plants, stem diameter, weight of shoots and shoot to root ratio, but not for weight of whole plants, and it increases weight of roots.

• The interaction between container size and irrigation frequency only affects shoot and root growth for stem diameter, weight of whole plants and weight of shoots.

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#### Table IV.

Effect of irrigation frequency and container size on the weight of shoots and shoot to root ratio of 'Rough Lemon' seedlings grown in Maseno, Kenya.

| Container volume (L)   | Irrigation frequency <sup>1</sup> | Weight of shoots (g) | Shoot to root<br>ratio |
|--|-----------------------------------|----------------------|------------------------|
| 1.7 (V <sub>1</sub> )  | W <sub>1</sub>                    | 26.9                 | 1.30                   |
|  | W <sub>2</sub>                    | 63.2                 | 1.00                   |
|  | W <sub>3</sub>                    | 17.2                 | 0.40                   |
| 2.7 (V <sub>2</sub> )  | W <sub>1</sub>                    | 78.0                 | 1.60                   |
|  | W2                                | 35.0                 | 0.90                   |
|  | W <sub>3</sub>                    | 63.4                 | 0.80                   |
| 4.5 (V <sub>3</sub> )  | W <sub>1</sub>                    | 125.2                | 0.70                   |
|  | W <sub>2</sub>                    | 87.4                 | 0.70                   |
|  | W <sub>3</sub>                    | 51.3                 | 0.69                   |
| Statistical parameter  |                                   | Weight of shoots (g) | Shoot to root<br>ratio |
| LSD <sup>2</sup> between irrigation frequency means                                    |                                   | 27.1                 | 0.32                   |
| LSD <sup>2</sup> between container sizes   |                                   | 35.7                 | 0.46                   |
| LSD <sup>2</sup> [container size × irrigation frequency]                               |                                   | 39.0                 | 0.25                   |
| Significance of <i>F</i> tests <sup>3</sup> for container sizes                        |                                   | Not significant      | Not significant        |
| Significance of $F$ tests <sup>3</sup> for irrigation frequency                        |                                   | Significant          | Not significant        |
| Significance of $F$ tests <sup>3</sup> for<br>[container sizes × irrigation frequency] |                                   | Significant          | Not significant        |

<sup>1</sup> W<sub>1</sub>: every day, W<sub>2</sub>: every 2 days, W<sub>3</sub>: every 3 days.

<sup>2</sup> LSD at  $P \le 0.05$ .

<sup>3</sup> F test at  $P \leq 0.05$ .

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### La contención de las raíces y la frecuencia de riego afectan el crecimiento de las plantas jóvenes de 'Rough lemon' (*Citrus limon*).

Resumen — Introducción. Una contención de las raíces tiene lugar cuando las plantas jóvenes se desarrollan en contenedores de tamaños pequeños, lo que reduce el crecimiento de las plántulas de distintas maneras. Los objetivos de nuestro estudio consistieron consecuentemente en estudiar los efectos del tamaño de los contenedores y los de la frecuencia de riego en parámetros de crecimiento de plantas de 'Rough lemon' (Citrus limon) observadas en un vivero en Maseno, Kenia. Material y métodos. Los tratamientos testaron el efecto de tres volúmenes de contenedor (1,7 L, 2,7 L y 4,5 L) y el de tres frecuencias de riego [diaria (W1), cada 2 días (W2) y cada 3 días (W3)]. Se seleccionó completamente de modo aleatorio el dispositivo experimental, que presentaba tres repeticiones. Los parámetros de crecimiento estudiados fueron la altura de la planta, la altura del follaje, el peso del tronco y el de las raíces, el número de hojas y la relación [tronco / raíces]. Resultados y discusión. El aumento del volumen de los contenedores permitió aumentar generalmente el tamaño de las plantas destinadas a la plantación y el del follaje, así como el peso de la planta entera y el de las raíces, pero no afectó el número de hojas, ni el diámetro del tronco, ni tampoco la relación [tronco / raíces]. La frecuencia de riego permitió aumentar el número de hojas, el tamaño de las plantas destinadas a la plantación y el del follaje, el diámetro del tronco y el peso de los troncos y de las raíces, pero este factor no tuvo ningún efecto en el peso de las plantas enteras. Estos resultados se explican debido a un mejor desarrollo de las raíces en los contenedores de volúmenes más grandes. Conclusión. Al aumentar el volumen de los contenedores de 1,7 L a 4,5 L; y, la frecuencia de riego de un riego cada 3 días a un riego diario, resultó mejorarse también el crecimiento de las plantas, expresado por distintos parámetros de crecimiento.

Nigeria / *Citrus limon* / propagación de plantas / viveros / plantón de vivero / crecimiento / plantación en contenedor / riego

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