Nitrogen-15 uptake and distribution in two citrus species

Rodrigo Marcelli Boaretto[A]; Dirceu Mattos Junior[A], José Antonio Quaggio[B]; Heitor Cantarella[B] and Paulo César Ocheuze Trivelin[C]

[A]Instituto Agronômico de Campinas – Centro de Citricultura, Cordeirópolis-SP, Brazil, Email boaretto@iac.sp.gov.br; ddm@iac.sp.gov.br
[B]Instituto Agronômico de Campinas – Centro de Solos, Campinas-SP, Brazil, Email quaggio@iac.sp.gov.br, cantarella@iac.sp.gov.br)
[C]Universidade de São Paulo – CENA, Piracicaba-SP, Brazil, Email pcotrive@cena.usp.br)

Abstract
Fruit yield of citrus is largely dependent on nitrogen fertilization, which it plays an important role in tree nutrition. An experiment was carried out in lysimeters (320 L), with 3-yr-old 'Valencia' sweet orange [*Citrus sinensis* (L.) Osbeck] and 'Lisbon' lemon (*C. limon* L.) trees grafted on 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.] and fertilized with ^{15}N-enriched urea in different periods. The objectives of this study were (i) to evaluate the N partitioning for the biomass content of orange and lemon trees, (ii) to estimate the ^{15}N uptake and recovery from labeled fertilizer applied to the soil in spring and summer periods, and (iii) to evaluate the nitrogen use efficiency by trees. The results showed that the dry mass accumulated in the lemon trees (6,499 g) was greater than in the orange trees (4,546 g). Total N concentration in the orange tree components was higher than in the lemon tree components. Total N fertilizer recovery was 36% for the orange trees and 52% for the lemon trees, for the average of the periods. The content of N derived from the fertilizer in the orange trees (74.6 g) was lower than for the lemon trees (98.6 g). Orange trees were less efficient in N use than the lemon trees.

Key Words
Orange, lemon, N fertilization, N absorption, N isotope, N use efficiency.

Introduction
The Brazilian citriculture has led world citrus production during recent years; the 20.8 million tonnes of fruit in 2007 represented about 19.1% of the overall world production (FAO 2009). Efficient nutrient management of citrus groves is critical to achieve high yields and crop quality. Nitrogen (N) is a key nutrient for production of citrus fruits, and the element plays an important role in vegetative and reproductive growth (Alva and Paramasivam 1998; Cantarella *et al.* 2003). The response of trees to N fertilization is likely to be influenced by endogenous N pools in the tree, N rates and application timing, sources of applied N, soil water availability, as well as soil N processes such as nitrification, denitrification, immobilization, leaching and volatilization (Martinez *et al.* 2002).

Soil analysis in Brazil has not been used for N recommendation for citrus, because it fails to correlate with fruit yield (Mattos Jr. *et al.* 1995). Leaf N analysis is a valuable tool for diagnosis of citrus tree nutrition and can also serve to predict N requirement (Quaggio *et al.* 1998). Adequate N content in the leaf for orange trees [*Citrus sinensis* (L.) Osb.] is around 27 g/kg (Quaggio *et al.* 1998), whereas for lemon trees [*C. limon* (L.) Burm. f.] a content higher than 18 g/kg does not indicate a positive effect of N fertilization on fruit yield (Quaggio *et al.* 2002). Adjustment of N fertilization recommendations for citrus, based on N dynamics in the tree is necessary to increase N uptake efficiency and minimize N losses in the soil-plant system (Syvertsen and Smith 1996). The aim of the work was (i) to evaluate the N partitioning in the biomass content of orange and lemon trees, (ii) to estimate the ^{15}N uptake and recovery from labeled fertilizer applied to the soil in spring and summer periods, and (iii) to evaluate the nitrogen use efficiency by the trees.

Methods
The experiment was carried out with 3-yr-old trees of 'Valencia' sweet orange [*Citrus sinensis* (L.) Osbeck] and 'Lisbon' lemon (*C. limon* L.) both grafted on 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]. The trees were the same size in the beginning of the study and were grown outdoors individually in lysimeters of 320 L filled with a clayey soil with, in g/kg , clay = 613 and sand = 161; nutrients were supplied according to Quaggio *et al.* (2005) and water was managed with aid of tensiometers and applied by drip irrigation.

The treatments were set up in a complete randomized factorial design of the type 2 x 2 (two species – orange and lemon; and two ^{15}N labelled applications – spring and summer) with three replicates. The annual N rate was 160 g/tree, which was split into two applications of 80 g/tree, in the spring and the same in the middle of the summer. The trees were ^{15}N fertilized with urea enriched in ^{15}N (2.1 atom% excess) just once either in the spring or in the summer. To avoid losses of N-NH_3 by volatilization, the urea was dissolved in water and applied to the soil surface; after the fertilization the lysimeters were irrigated to incorporate the fertilizer.

As soon as the fruits were mature, the trees were destructively harvested and separated into different components: new branches (<1.5 cm Ø) + leaves; old branches (1.5-4.0 cm Ø) + leaves; woody branches (>4.0 cm Ø); roots, mature fruits (last blooming) and new fruits (+/- 2.0 cm Ø). Tree components were weighed and sampled for determination of dry mass, N concentration and isotopic ratio.

Total N concentration and $^{15}N/^{14}N$ ratio were determined according to Barrie and Prosser (1996). The percentage of N in the plant components derived from the fertilizer (Ndff) and the total amount of N recovered (fertilizer N recovery) in different plant components were calculated using the isotopic dilution equation described by Hauck and Bremner (1976). The amount of ^{15}N recovered by citrus trees was calculated based on dry mass, total N and Ndff determination for the tree components.

Results

Dry mass, as well as total N concentration and N content of different tree components in both species are presented in Table 1. In the beginning of the experiment, orange and lemon trees had similar sizes; however, when the trees were destructively harvested, the dry mass of the lemon trees was 42% higher than that of orange trees. The largest proportion of total tree dry mass was found in the root, which represented 31% in the lemon and 40% in the orange trees. The lowest total N concentration was in the branches (woody, old and new) and the highest values were found in the leaves. N concentration in the orange tree components was higher than in the lemon trees. However, the total N content in the lemon trees was greater than in the orange ones, because of the total dry mass of the lemon trees was greater.

Table 1. Biomass distribution, total N concentration and N content in orange and lemon tree parts (values are means of 6 replicates ± standard deviation).

Plant part	Dry mass		N Concentration		N Content	
	Orange	Lemon	Orange	Lemon	Orange	Lemon
	g ±s(m)[A]		(g/kg) ±s(m)		g	
Young fruits	440±115	1233±379	14.6±1.9	13.9±1.9	6.5	17.2
Mature fruits	355±136	181±218	16.0±0.8	12.8±0.8	5.7	2.9
New leaves	630±123	757±103	28.2±1.5	22.6±1.5	17.7	17.1
Old leaves	105±32	208±82	27.1±1.1	23.6±1.1	2.8	4.8
New branches	325±95	475±118	10.9±1.0	7.2±1.0	3.6	3.4
Old branches	252±51	507±125	7.0±0.5	5.0±0.5	1.8	2.4
Woody branches	581±160	1108±227	5.4±0.3	4.2±0.3	3.1	4.5
Roots	1858±424	2030±260	17.7±2.5	22.9±2.5	33.4	46.3
TOTAL	4546±371	6499±445	(16.4)[B]	(15.2)[B]	74.6	98.6

[A]s(m) = the standard error of the mean, n = 6.
[B]Average in the whole tree

Nitrogen use efficiency is defined as the dry mass produced by unit nitrogen content of trees. Nitrogen concentration in the whole lemon tree (15.2 g/kg) was lower than in the whole orange tree (16.4 g/kg) (Table 1). It suggested that the lemon tree was more efficient in the use of N than the orange. Nitrogen uptake and fate of the labeled nutrient in the components of the citrus trees are shown in Table 2. The highest content of N uptake from fertilizer was found in the roots and new leaves. Tree N labeled period in the spring (220 days) was longer than the ^{15}N period in the summer (120 days); consequently, the content of ^{15}N in the trees that were fertilized in the spring was higher than those in the summer. The content of N derived from fertilizer in the lemon trees was higher than the orange trees, due to the higher ^{15}N recovered by the roots and the young fruits. Mattos Jr. *et al.* (2007) reviewed several experiment results in which the labeled ^{15}N was used; it was found that N recovered from urea by citrus trees in different production systems, ranged from 20 to 50%. In the present experiment, the total N recovery in the plant soil system was about 36% for orange trees and 52% for lemon trees at the end of the growth cycle (Table 2).

Table 2. Content of nitrogen derived from fertilizer (Ndff) in orange and lemon tree parts and N recovery of the labeled fertilizer in different fertilization periods (values are means of 3 replicates ± standard deviation).

	Spring		Summer	
	Orange	Lemon	Orange	Lemon
	g Ndff ±s(m)[A]			
Young fruits	3.53±0.8	7.99±1.9	1.62±0.5	6.33±1.7
Mature fruit	2.19±1.3	2.08±0.9	0.60±0.1	0.18±0.03
New leaves	8.22±3.1	7.93±2.0	5.58±1.9	5.78±1.6
Old leaves	1.10±0.6	1.65±0.5	0.63±0.3	1.38±0.5
New branches	1.57±0.5	1.54±0.3	1.36±0.6	1.44±0.5
Old branches	0.75±0.1	1.12±0.1	0.60±0.2	0.68±0.2
Woody branches	1.60±0.6	1.84±0.4	0.89±0.3	1.49±0.7
Roots	15.41±4.1	18.91±5.3	12.10±4.8	22.91±6.7
TOTAL	34.36	43.05	23.38	40.19
			%	
N recovery (relative to the total)	42.9	53.8	29.2	50.2

[A]s(m) = the standard error of the mean, n = 3.
Trees destructively harvested 220 days after spring application of ^{15}N.
Trees destructively harvested 120 days after summer application of ^{15}N.

Approximately 40% of the N in mature fruits was derived from the spring fertilization, even though only 10% was derived from the summer fertilization. On the contrary, the summer fertilization had a higher N contribution to the flowering in the next season (Table 3). Specific leaf weight is defined as leaf mass per unit area, and it is directly proportional to leaf thickness. By the results shown in the Figure 1A, the specific orange leaf weight was greater than the lemon leaf weight; this suggests that the lemon leaf is thinner than the orange leaf. In addition, the N concentration in the orange leaf was higher than in the lemon leaf, consequently, the specific orange leaf nitrogen, which is defined as leaf N content per unit leaf area (g m^{-2} of N), was much higher than the specific lemon leaf nitrogen (Figure 1B).

Table 3. Nitrogen derived from the labeled fertilizer (Ndff) in flowers and fruits of orange and lemon trees in the different fertilization periods (values are means of 3 replicates ± standard deviation).

	Spring		Summer	
	Orange	Lemon	Orange	Lemon
	% Ndff ±s(m)[A]			
Mature fruit	37.6±3.9	41.9±6.9	10.3±2.4	10.7±0.4
Young fruits (2 cm Ø)	44.1±5.5	42.4±7.7	33.1±5.8	36.2±5.1
Flowers	37.9±6.9	33.0±6.4	40.9±7.9	51.5±2.6

[A]s(m) = the standard error of the mean, n = 3.
Fruit harvested 220 days after spring application of ^{15}N.
Fruit harvested 120 days after summer application of ^{15}N.

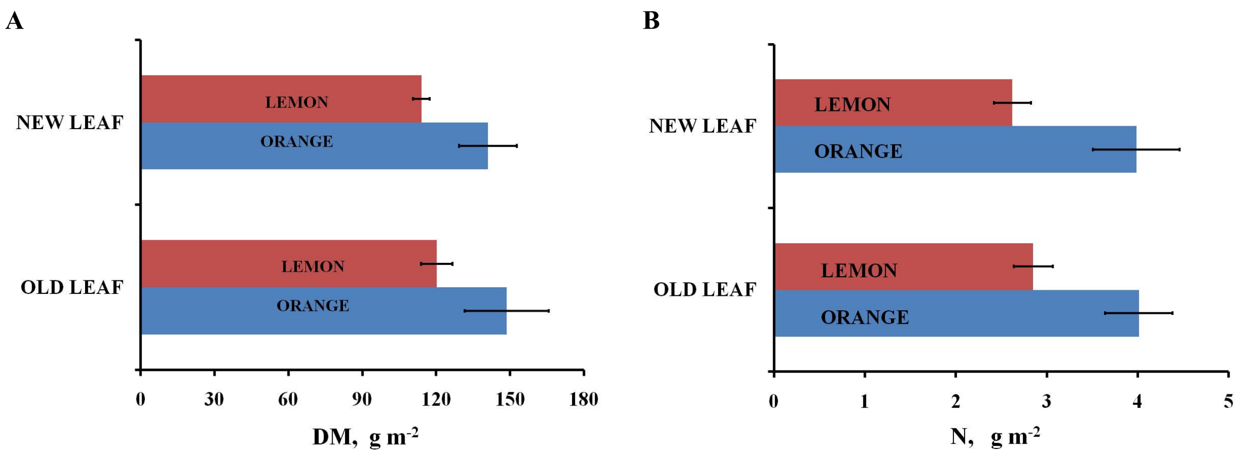

Figure 1. Specific leaf weight and specific leaf nitrogen (bars are the standard error of the mean, n=3).

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