Biomass Distribution and Nitrogen-15 Partitioning in Citrus Trees on a Sandy Entisol

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ABSTRACT

The area under citrus production in Florida is 332 000 ha, with a production of 10 to 12 million metric tonnes of fruit annually. Nutrient management recommendations are needed to increase N uptake efficiency and to minimize nitrate leaching below the root zone. The objectives of this study were (i) to evaluate biomass distribution of 6-yr-old 'Hamlin' orange trees [*Citrus sinensis* (L.) Osbeck] on 'Swingle citrumelo' [*Poncirus trifoliata* (L.) Raf. × *C.* × *paradisi* Macfad.] rootstock grown in a sandy soil under low volume irrigation, and (ii) to estimate partitioning of ^{15}N fertilizer applied to the soil during early spring into different tree components. We evaluated biomass of tree components (leaves, twigs, trunk, taproot, roots, and fruit), and N recovery and distribution of $^{15}NH_4{}^{15}NO_3$ (AN) and ^{15}N-urea (UR) (10 atom % ^{15}N) applied to the soil surface. About 70% of dry matter biomass of trees was aboveground (AG). Length density of feeder roots was concentrated at a depth of 0 to 15 cm below the soil surface and varied from 1.87 to 0.88 cm cm^{-3} at 0.5- and 1.5-m distance from the trunk, respectively. Total recoveries of ^{15}N by trees were 25.5% for UR and 39.5% for AN at fruit harvest, 280 d after fertilization. Mean accumulation of applied ^{15}N in recent leaf flush was 4.2% and that of older leaves was 2.5%. Accumulation of ^{15}N was low in woody tissue. Since fruit represented a large sink for N (10.2 and 18.4% recovery of ^{15}N applied as UR and AN, respectively), we confirmed the importance of N fertilization before fruit development.

Best management practices (BMPs) for citrus have been proposed to improve the efficiency of N utilization for high fruit yield and also to address environmental quality issues regarding N contamination of ground water in Florida. Nitrogen fertilization and irrigation are two important cultural factors responsible for optimal production.

Perennial trees store large quantities of N within various plant components, which can be utilized for tree growth and fruit yield in subsequent seasons, as reported for almond trees {*Prunus amygdalus* Batsch [= *P. dulcis* (Mill.) D.A. Webb]} (Weinbaum et al., 1984), citrus (Legaz et al., 1995), kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson] (Ledgard and Smith, 1992), and apple (*Malus domestica* Borkh.) (Millard and Neilsen, 1989; Khemira et al., 1998). The annual vegetative growth and the fruit yield of citrus trees contain a variable proportion of the fertilizer N applied during the growth period. A great amount of N in the new growth may be drawn from the tree biomass. Therefore, the N reserve in the leaves and structural components plays an important role in the development of new flushes of growth and flowers in the spring (Kato, 1986).

Nitrogen distribution in 'Valencia' orange trees, grown in sand culture and fertilized with a nutrient solution labeled with ^{15}N, presented distribution of labeled N among tree parts ranked in the order: leaves (49.7%) > roots (19.2%) > twigs and stem (14.3%) > flowers (10.6%) > ovaries (6.1%) (Legaz et al., 1981). The mobilization of N reserves in oranges is a result of biochemical processes, in which total protein content of old leaves decreases progressively beginning in the spring. The mobilized protein comes from an aqueous protein that is the major fraction of the total leaf protein (Moreno and Garcia-Martínez, 1984).

The removal of N in harvested plant biomass has been used to estimate N requirements in some crops, particularly annual grain crops. In contrast, the large pool of N present in the structural components of citrus trees implies that distribution and remobilization of N within the tree play an important role in determining the N requirement on an annual basis which must be known to maximize N uptake efficiency and minimize its losses (Sanchez et al., 1995).

Efficient management of irrigation can minimize leaching losses of highly soluble nutrients (i.e., nitrate) through the soil profile below the rooting zone. Citrus production in deep sandy soils with high volume irrigation systems, as used in central Florida in the past, tends to cause the upper soil layers to dry between long irrigation intervals. This condition favors deep rooting, as reported by Cahon et al. (1962), Castle and Krezdorn (1977), Castle et al. (1993), and Boman et al. (1999). The same occurs in nonirrigated groves, where the majority of the root system can reach depths > 1.5 m in the soil (Pace and Araujo, 1986; Oliveira et al., 1998). However, using low volume irrigation systems to replenish the moisture deficit in the surface soil may lead to a shallow rootzone of the citrus trees. Since citrus growth and root distribution can be modified as a result of changes in the root environment, a clear understanding of the root system is also important to develop irrigation and nutrient BMPs in an effort to improve uptake efficiency and minimize losses below the rooting depth.

The objectives of this study were (i) to evaluate biomass distribution of 6-yr-old citrus trees grown in a sandy soil under low volume irrigation, and (ii) to estimate partitioning of soil-applied–^{15}N during early spring in different plant components during fruit harvest.

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Abbreviations: AG, aboveground; AN, ammonium nitrate; BG, belowground; BMP, best management practice; DAF, days after fertilization; Ndff, N in plant components derived from the labeled fertilizer; PVC, polyvinyl chloride; UR, urea.

MATERIALS AND METHODS

Site Characteristics and Treatments

A citrus grove was planted with Hamlin orange trees on Swingle citrumelo rootstock. Trees were planted in September 1993 at 7.6- by 4.6-m spacing (285 trees ha^{-1}) on a Candler fine sand (hyperthermic, uncoated Typic Quartzipsamments; sand = 967 g kg^{-1} in the top 15 cm), having a pH of ≈7.0, and CEC of 2.2 $cmol_c$ kg^{-1} within the 0- to 30-cm depth. This grove was managed with application of dry soluble fertilizer containing N, P, and K. The annual rate of N was equivalent to 230 g N $tree^{-1}$ as AN. Trees were irrigated using one undertree low volume emitter per tree covering ≈7 m^2 with a delivery rate of 50 L h^{-1}. Irrigation was initiated based on 33% depletion of the available soil moisture within the 40-cm soil depth determined by a multisensor capacitance probe (Enviro-SCAN, Sentek PTY Ltd., South Australia) placed at the drip line (Alva and Fares, 1998; Fares and Alva, 1999).

The experiment, a randomized complete block design with two treatments (N source) and three replications (with one tree each), was initiated in Feb. 1999 and continued until Dec. 1999. Fertilizer sources included: (i) UR and (ii) AN. The labeled fertilizers had an isotopic enrichment of 10 atom % ^{15}N. Fertilizers were uniformly distributed as dry granules to the soil surface in a circular area (1.10-m radius) under the tree canopy. Twenty-five percent of the recommended annual N rate of 230 g $tree^{-1}$ (Ferguson et al., 1995) was applied as labeled N on 15 Feb. 1999. Following fertilizer application, the area received 7 mm of irrigation water to promote fertilizer dissolution and shallow incorporation into the soil. The grove irrigation was then managed as described earlier.

Two additional applications of nonlabeled N were made on 8 June and 9 Sept. 1999 (in equal amounts of 86 g $tree^{-1}$) to supply the trees with the remaining recommended annual N rate. Phosphorus and K were applied at 18 g P $tree^{-1}$ and 200 g K $tree^{-1}$, also in June and September.

Tree Sampling, Biomass Estimation, and Isotopic Determination

Tree flowering started in late February 1999 and the spring flush had completely expanded leaves in April 1999. Leaves and fruit were collected in the experiment following fertilizer application in February 1999. The mature flush of leaves and the summer/fall 1998 leaf flush were sampled at 0 (day of fertilization), 7, 14, 21, 28, 35, 49, 63, and 77 d after fertilization (DAF). The summer/fall 1998 leaf component was also sampled at 113 and 206 DAF. The 1999 spring flush leaves were sampled at 49, 63, 77, 113, and 206 DAF. Leaf samples on each sampling date comprised 10 leaves per tree. Fruit samples were taken in April (63 DAF; 20 per tree), June (113 DAF; 10 per tree), and September 1999 (206 DAF; 10 per tree), when they had an average diameter of 1.5, 3.5, and 5.0 cm, respectively. Leaves and fruit were washed in detergent solution and thoroughly rinsed in tap water, followed by distilled water, and then dried at 65°C for 72 h (fruit were sliced in small pieces before drying). The dried tissue was ground to pass through a 0.635-mm screen using a ball mill. Grinding containers were washed with 0.2 mol L^{-1} H_2SO_4 solution and rinsed with deionized water between samples.

The concentration of N and the N isotopic ratio of tissue material were determined with an automated Roboprep C/N analyzer linked to a Tracer Mass Isotope Ratio Mass Spectrometer (Europa Scientific, Ltd., Cheshire, UK) at the Stable Isotope Research Unit, Oregon State University. The tracer mass system is capable of analyzing with a precision of 0.8 atom ‰ ^{15}N for levels above natural abundance.

Trees that received AN fertilizer (one per plot) were destructively harvested in Dec. 1999 for evaluation of dry mass distribution in different tree components and sampled for determinations of N concentration and isotopic ratio as described above. The AG portion was divided into (i) summer/fall 1999 leaf flush; (ii) spring 1999 plus older leaves (the latter component was most made up by summer/fall 1998 flushes); (iii) twigs >1.5 cm in diameter; (iv) twigs ≤1.5 cm in diameter; (v) trunk; and (vi) fruit. Soil was excavated in two opposing quadrants (NW and SE) of 1.75 ×1.75 m each, marked on the soil surface, and which had the tree trunk as a common vertex. Then, roots removed from 0- to 15-, 15- to 30-, and 30- to 45-cm depths were separated from soil with a 0.2-cm mesh sieve into the following size classes: (i) fibrous roots (<0.2-cm diam.), (ii) woody roots (0.2- to 1.0-cm diam.), and (iii) woody roots (>1.0-cm diam.). The taproot was also separated from the soil and together with the roots, comprised the belowground (BG) portion of the tree.

Samples from tree components were collected in the field and placed in sealed plastic bags to prevent water loss, then weighed in the field. Later, the same material was washed in the laboratory and dried in an oven (65°C; 72 h) for dry mass determination and further N analysis as described earlier. Woody tissue samples were cut in small pieces (<10-mm), ground to pass through a 100-mm screen using a rotary mill, and then to pass a 0.635-mm screen using a ball mill. Total dry mass of roots was estimated by multiplying the values obtained for both excavated soil quadrants by two. Juice quality of fruit subsamples was determined (total soluble solids, citric acid content, and soluble solids/acid ratio) according to standard procedures (Wardowski et al., 1995).

Trees that received the UR fertilizer also had the AG portion destructively harvested. Dry mass of components was determined based on the moisture content of samples collected in the field and dried in an oven (65°C; 72 h). Root excavation was not done from this treatment, since we assumed that root distribution for UR-treated trees would be similar to that of AN-treated trees. Nitrogen concentration and N isotopic ratios were determined for each tree part collected.

The percentage of N in the plant components derived from the labeled fertilizer (Ndff) and the total amount of N recovered (fertilizer N recovery) in different plant components were calculated using the isotopic dilution equations described by Hauck and Bremner (1976).

Root Distribution

Roots of AN-treated trees were also sampled in December 1999, before destructive harvest, using a 5-cm diam. polyvinyl chloride (PVC) corer for root density and average root diameter estimations. Samples were collected at 0- to 15-, 15- to 30-, and 30- to 45-cm soil depths every 50, 100, and 150 cm from the tree trunk in the N-S (within row), and E-W (between rows) directions. Samples were taken to the laboratory in sealed plastic bags and separated with a 0.2-cm mesh sieve. Two classes of root size were separated using forceps: (i) <0.2 cm in diameter and (ii) 0.2 to 1.0 cm in diameter. The roots were cleaned and fresh mass was recorded. Root length was determined by counting the number of horizontal and vertical intersections of roots in a grid system of 1.0 × 1.0 cm (Tennant, 1975), which multiplied by 11/14 and divided by the volume of the PVC corer (295 mL) gives the root density in cm cm^{-3} soil. Mean root radius (r_0) was calculated assuming that fresh roots have a density of 1 Mg m^{-3} by the equation $r_0 = (F_{wr}/\pi L)^{1/2}$, where F_{wr} is the fresh mass of roots (in grams), and L is the total root length (in centimeters) (Barber, 1995).

Table 1. Mean dry mass distribution of 6-yr-old 'Hamlin' orange tree on 'Swingle citrumelo' rootstock fertilized with NH_4NO_3.

Tree component	DM†	SE‡	AG§	BG¶	Total#
	g		%		
Summer + fall 1999 leaf flush	1 567	211	8.7		6.3
Spring 1999 + older leaf flush	847	116	4.7	–	3.4
Twigs > 1.5-cm diam.	2 267	317	12.5	–	9.1
Twigs ≤ 1.5-cm diam.	4 249	760	23.5	–	17.0
Trunk	1 566	105	8.7	–	6.3
Fruit (9-mo-old)	7 580	202	41.9	–	30.3
			0- to 15-cm soil depth		
Fibrous roots (≤0.2-cm diam.)	1 771	66	–	25.5	7.1
Woody roots (0.2- to 1.0-cm diam.)	565	55	–	8.1	2.3
Woody roots (≥1.0-cm diam.)	461	314	–	6.6	1.8
			15- to 30-cm soil depth		
Fibrous roots (≤0.2-cm diam.)	611	219	–	8.8	2.4
Woody roots (0.2- to 1.0-cm diam.)	413	188	–	5.9	1.6
Woody roots (≥1.0-cm diam.)	271	137	–	3.9	1.1
			30- to 45-cm soil depth		
Fibrous roots (≤0.2-cm diam.)	87	55	–	1.3	0.3
Woody roots (0.2- to 1.0-cm diam.)	50	28	–	0.7	0.2
Woody roots (≥1.0-cm diam.)	55	50	–	0.8	0.2
Taproot	2 667	827	–	38.4	10.7
Total AG	18 076	349	100.0	–	–
Total BG	6 952	580	–	100.0	–
Grand Total	25 028	482	–	–	100.0

† Dry mass of tree component.
‡ The standard error of the mean (n = 3).
§ Proportion of the aboveground dry mass.
¶ Proportion of the belowground dry mass.
# Proportion of the total dry mass.

Data Analysis

Standard deviations were calculated for mean Ndff, dry matter distribution of tree components, and average root density for each soil depth and trunk distance. A simple analysis of variance was used to test the hypothesis that means from AG biomass distribution of fertilized trees, root distribution obtained for soil quadrants, N content, ^{15}N enrichment, and ^{15}N recovery of tree components were equal (P = 0.05) using the GLM procedure of the SAS system (SAS Institute, 1996).

RESULTS AND DISCUSSION

Tree Biomass Distribution

Fertilized trees were destructively harvested when fruit reached maturity. The mean height of the trees was 2.5 ± 0.01 m with a canopy diameter of 2.5 ± 0.14 m. Mean trunk height was 41.4 ± 3.9 cm, and circumference was 24.5 ± 1.5 cm above the bud union. The AG dry mass estimated for the AN or UR fertilizer treatments was >70% of the total tree biomass (Tables 1 and 2).

The largest proportion of total tree dry mass was that of fruit, which represented 28 to 30%. The average fruit yields were 52.43 ± 3.98 kg tree^{-1} for the AN and 51.42 ± 3.99 kg tree^{-1} for the UR treatments (fresh mass basis). The N source effect was nonsignificant on the fruit yield. The juice analysis of fruit samples taken in Dec. 1999 showed total soluble solids = 10.5 ± 0.07° Brix, titratable acidity = 0.69 ± 0.04% (w/v) and soluble solids/acid ratio = 15.3 ± 1.14. Since trees were uniformly fertilized before labeled fertilizer application, it is often difficult to verify growth differences in a single year (Sanchez et al., 1995).

Leaves accounted for ≈13% of the AG biomass. The summer plus fall 1999 flush represented the major portion of total leaf dry mass (Tables 1 and 2). Stansly et al. (1996) reported the seasonal growth pattern of 4-yr-old grapefruit (*C.* × *paradisi* Macfad.) trees, in which ≈20% of leaf area at the end of a growing season was carryover from the previous year and only 25% came from spring flush compared with 33% in summer and 35% in fall. This can be expected due to a larger size of individual leaves (summer + fall 1999 flush) compared with mature or spring flush leaves.

Roots accounted for 27.7% of the total dry mass of the tree. Fibrous roots accounted for the greatest proportion in the BG portion (35.6%) after the taproot (38.4%) (Table 1). More than 70% of fibrous roots were found in the 0- to 15-cm depth, and at deeper depths the root distribution data showed substantial variation as evident from greater coefficients of variation (Table 1). Woody roots followed a similar pattern as de-

Table 2. Mean biomass distribution of 6-yr-old 'Hamlin' orange tree on 'Swingle citrumelo' rootstock fertilized with urea.

Tree component	DM†	SE‡	AG§	Total¶
	g		%	
Summer + fall 1999 leaf flush	1660	114	8.8	6.5
Spring 1999 + older leaf flush	896	61	4.8	3.5
Twigs > 1.5-cm diam.	2396	164	12.8	9.3
Twigs ≤ 1.5-cm diam.	4481	307	23.9	17.4
Trunk	2037	468	10.9	7.9
Fruit (9-mo-old)	7297	596	38.9	28.4

† Dry mass of tree component.
‡ The standard error of the mean (n = 3).
§ Proportion of the aboveground dry mass.
¶ Proportion of the total dry mass, assuming the biomass of roots is similar to that of the AN-fertilized trees.

scribed above, and represented <26% of the total root system (as in the BG portion) within the 0- to 45-cm soil depth layer. Dasberg (1987) reported that the AG portion of 9- to 20-yr-old citrus trees including leaves, fruit, trunk, and branches accounted for >65% of the tree biomass. The dry matter partitioning of 22-yr-old 'Shamouti' orange trees (Feigenbaum et al., 1987) was: branches and twigs, 30.1%; trunk plus small branches, 25.3%; roots, 24.0%; fruit, 13.3%; and leaves, 7.3%. In the case of nonbearing (32-mo-old) Hamlin trees roots, trunk, large branches, leaves, and small branches accounted for 28.1, 26.1, 21.2, 18.0, and 7.8%, respectively, of the tree biomass (Alva et al., 1999). Our data are comparable to the above values since >70% of the total tree biomass was found in the AG components. Proportions of the dry mass of trunk or leaves deviate from values reported by Feigenbaum et al. (1987) and Alva et al. (1999) since the former presented a total value for trunk and main branches, whereas the latter harvested trees with no fruit. Dry mass distribution in citrus trees varies with the whole tree N status and with fruit load, which determines a competitive allocation effect of biomass among tree components (Lea-Cox et al., 2001). Furthermore, Swingle citrumelo is a superior rootstock for sweet oranges for high fruit yields under irrigation (Wutscher and Bistline, 1988; Castle et al., 1993). Such high yields, when related to a resulted smaller canopy volume of trees, can explain why fruit are a greater proportion of tree biomass compared with the value reported by Feigenbaum et al. (1987).

Root Density Distribution

Trees had uniform fresh root length density (L_v, cm root cm^{-3} soil) in all four directions evaluated ($P > 0.05$). Root density was greater closer to the tree trunk on both horizontal and vertical planes ($P < 0.05$) (Fig. 1A and B). Root density decreased from 1.85 cm cm^{-3} at the 0- to 15-cm depth to 0.16 cm cm^{-3} at the 30 to 45-cm depth within 50 cm from the tree trunk. At 150 cm from the trunk, root density was 48% less than at the 50-cm distance from the trunk in the 15-cm soil depth (Fig. 1A). The same pattern was found for each soil layer evaluated. Our values of L_v approach those reported for apple trees in the top 1.0 m of soil, which ranged from zero to ≈1.0 cm cm^{-3} (Hughes and Gandar, 1993; De Silva et al., 1999).

In a sandy soil, using continuously monitored low volume irrigation with emitters positioned under the tree canopy, root growth is limited to the wetted soil volume (Spiegel-Roy and Goldschmidt, 1996). Zhang et al. (1996, 1998) found that root density (dry mass basis) was significantly greater near the emitter and at the 0- to 15-cm deep layer for grapefruit trees. Fibrous roots, with mean diameter of 0.08 ± 0.01 cm accounted for a major portion of the root density. Root density for large roots (0.2- to 1.0-cm diam.) was highly variable as indicated by the high standard deviation of means in Fig. 1B. Data presented points out the dynamic character of the citrus root system and its apparent adaptation to soil texture and irrigation (Alva and Tucker, 1997).

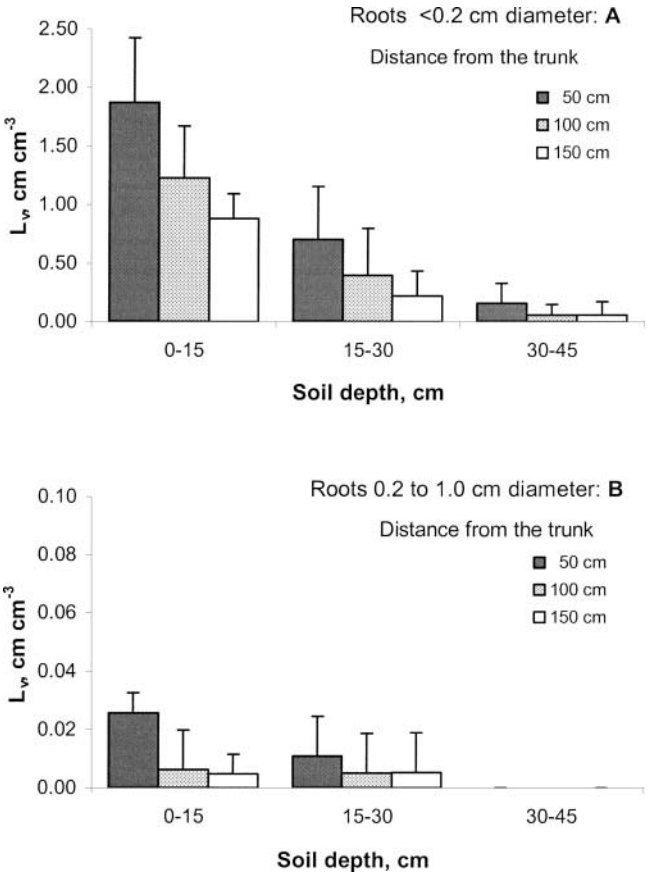

Fig. 1. Root length density (L_v) distribution of 6-yr-old 'Hamlin' orange trees on 'Swingle citrumelo' rootstock in a sandy Entisol. Vertical bars represent the standard error of the mean ($n = 12$). (A) Class of roots < 0.2 cm in diameter; (B) Class of roots between 0.2 and 1.0 cm in diameter.

Nitrogen-15 Taken up by Leaves and Fruit

Early effects of ^{15}N application were detected by evaluating percentages of Ndff in the leaves (Fig. 2A and 3A). Maximum values of Ndff observed were ≈40% (Fig. 2A), indicating the importance of other N sources (tree reserve and soil) for the growth of citrus trees. Such limited contribution of fertilizer-N was also observed by Sanchez et al. (1992) for established pear (*Pyrus communis* L.) trees. A larger proportion of N from either the UR or AN labeled fertilizers occurred in younger leaves (22 to 38%), especially for the spring 1999 flush, compared with mature leaves (7 to 12%) (Fig. 2A and 3A). The N remobilization process involves several biochemical steps of protein degradation and translocation into different tree components (Titus and Kang, 1982; Kato, 1986; Engels and Marschner, 1995). Such mobilized N may not be enough to support large N sinks, and then newly absorbed soil N appeared in new growing tissues. Kato et al. (1982) showed that in the coldest season, N uptake by 'Satsuma' mandarin (*C. reticulata* Blanco) trees was ≈10% of the amount taken up in summer. More than 90% of the applied N was found in the roots in the winter; on the other hand, in the summer, 55% of the absorbed N translocated upwards and most of it was found in the developing

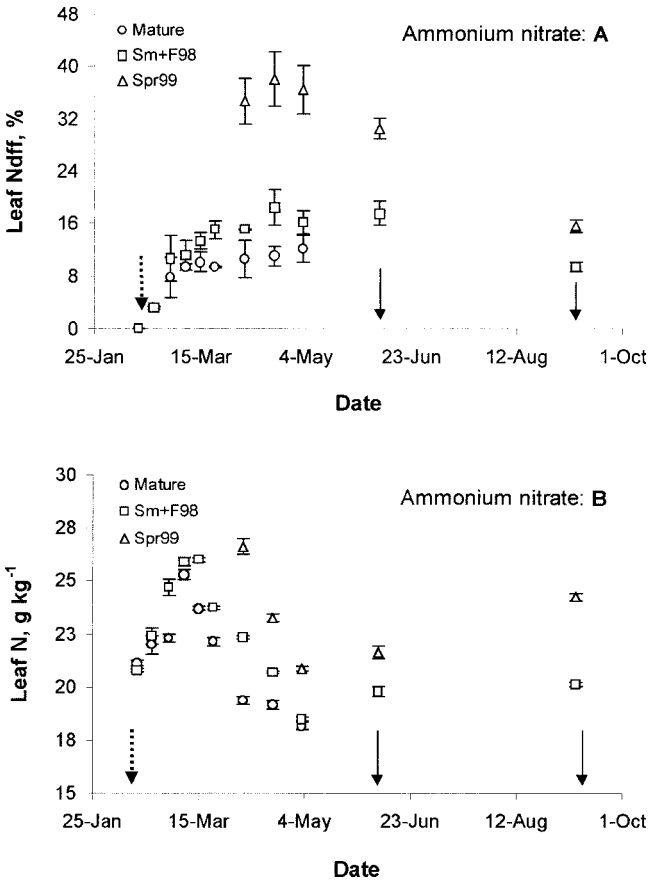

Fig. 2. Nitrogen in leaf samples derived from the labeled NH_4NO_3 fertilizer. Vertical bars are the standard error of the mean (n = 3). Mature = oldest leaf flush; Sm + F98 = summer and fall 1998 leaf flush; and Spr99 = spring 1999 leaf flush. Dashed arrow indicates time of ^{15}N application. Other arrows indicate further application of nonlabeled fertilizer. (A) Percentage of N derived from the labeled fertilizer; (B) leaf N concentration.

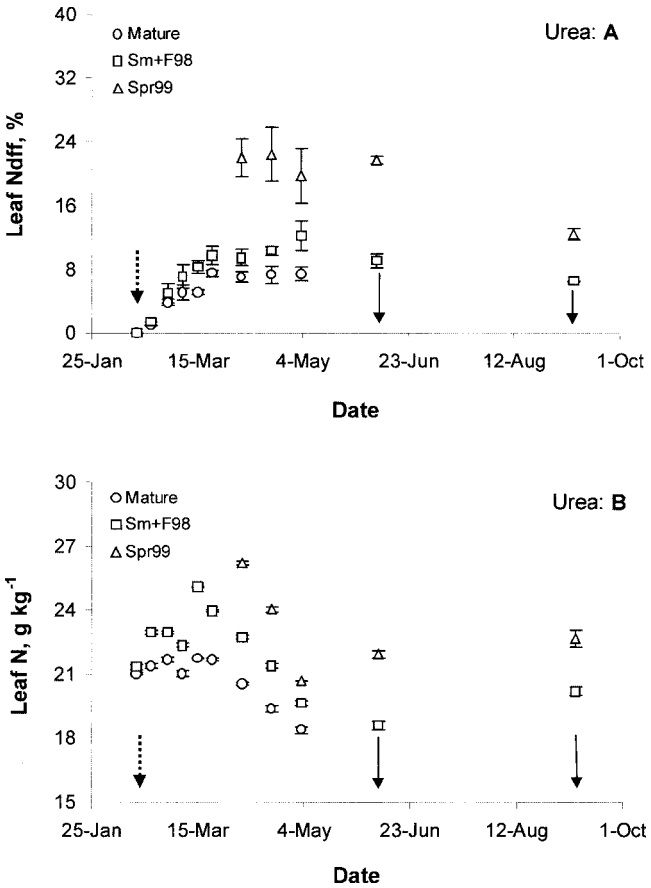

Fig. 3. Nitrogen in leaf samples derived from the labeled urea fertilizer after application. Vertical bars are the standard error of the mean (n = 3). Mature = oldest leaf flush; SM + F98 = summer and fall 1998 leaf flush; and Spr99 = spring 1999 leaf flush. Dashed arrow indicates time of ^{15}N application. Other arrows indicate further application of nonlabeled fertilizer. (A) Percentage of N derived from the labeled fertilizer; (B) Leaf N concentration.

new shoots. This suggests that the N taken up by the roots was translocated to the AG portion of the tree due to the high sink demand for N in protein synthesis of new developing organs (Legaz and Primo-Millo, 1984; Kato, 1986; Lea-Cox et al., 2001). Maximum root absorption efficiency is also reported to occur in late spring and early summer for peach trees [*Prunus persica* (L.) Bastch] (Muñhoz et al., 1993). The %Ndff increased gradually until 15 March for UR and 5 May for AN fertilized trees.

The observed difference between N sources is attributed to the NH_3 losses from applied UR fertilizer, and consequently, a lower availability of the nutrient for plant uptake. Another study conducted in this experiment (soil pH ≈7.0) following application of fertilizers demonstrated that volatilization accounted for 14.9 and 32.3% of applied N for AN and UR, respectively (Mattos, 2000). Since we assumed that tree biomass distribution was similar for UR and AN fertilized trees, the differences in %Ndff between treatments were probably due to differences in the amount of remaining N absorbed after fertilization. A plateau for Ndff was reached after 4 May, and the further decrease was probably associated with the uptake of nonlabeled N applied on 8 June and 9 September (Fig. 2A and 3A).

The total N concentration in the leaves of the orange trees before fertilization was ≈21 g kg^{-1} (Fig. 2B and 3B), and increased for 4 wk after application of the labeled fertilizer when levels of 26 g kg^{-1} for the summer/fall 1998 flush for both treatments were observed (Fig. 2B and 3B). Then N concentration declined after 15 March, probably as a result of combined processes of N redistribution from mature tissue and leaf expansion of young tissue (Calot et al., 1984). Nitrogen redistribution was evident since the concentration in mature leaves of AN-treated trees (25.3 g kg^{-1}) was higher than that of UR-treated ones (21.7 g kg^{-1}), whereas no major difference appeared for the summer/fall 1998 leaf flush as presented above. By 15 March, the residual soil inorganic ^{15}N was very low (data not shown) for significant uptake and maintenance of the Ndff proportions in the leaves.

Nitrogen-15 Recovery by Tree Components

Nitrogen concentration was lowest in the trunk and taproot (3.7 to 4.4 g kg^{-1}). The N concentration of twigs

Table 3. Average ^{15}N recovery of biomass components of 6-yr-old 'Hamlin' orange trees on 'Swingle citrumelo' rootstock calculated by isotopic dilution, 280 d after ^{15}N labeled NH_4NO_3 (AN) or urea (UR) fertilizer application.

Tree component	AN		UR		
	N recovery	SE†	N recovery	SE†	$P < F$
	^{15}N, % of applied				
Summer + fall 1999 leaf flush	5.49	0.64	3.22	0.20	0.0574
Spring 1999 + older leaf flush	3.08	0.23	1.85	0.21	0.0267
Twigs > 1.5-cm diam.	1.09	0.15	0.62	0.09	0.2431
Twigs ≤ 1.5-cm diam.	4.12	0.84	2.62	0.56	0.0641
Trunk	0.60	0.13	0.37	0.08	0.1814
Fruit (9-mo-old)	18.45	1.66	10.24	2.01	0.0148
			0- to 15-cm soil depth‡		
Fibrous roots (≤0.2-cm diam.)	3.67	0.56	2.25	0.13	0.0587
Woody roots (0.2- to 1.0-cm diam.)	0.35	0.04	0.33	0.03	0.6681
Woody roots (≥1.0-cm diam.)	0.26	0.20	0.23	0.20	0.8504
			15- to 30-cm soil depth‡		
Fibrous roots (≤0.2-cm diam.)	1.04	0.39	0.62	0.20	0.1766
Woody roots (0.2- to 1.0-cm diam.)	0.38	0.21	0.22	0.16	0.3540
Woody roots (≥1.0-cm diam.)	0.16	0.07	0.17	0.10	0.9479
			30- to 45-cm soil depth‡		
Fibrous roots (≤0.2-cm diam.)	0.13	0.08	0.07	0.04	0.2920
Woody roots (0.2- to 1.0-cm diam.)	0.03	0.02	0.03	0.01	0.9848
Woody roots (≥1.0-cm diam.)	0.03	0.02	0.03	0.03	0.8244
Taproot	0.58	0.08	0.65	0.08	0.7178
Total	39.45		25.53		0.0062

† The standard error of the mean ($n = 3$).
‡ Roots of UR-fertilized trees were not collected for determination of total dry mass. Recovery of ^{15}N was estimated based on the assumption that biomass of the root system was similar to that of AN-fertilized trees and on ^{15}N enrichment of root samples taken from the former.

(4.0 to 7.8 g kg^{-1}) and roots (5.8 to 17.0 g kg^{-1}) varied depending on the tissue age. Younger roots had greater N concentration compared with older roots. Nitrogen concentration in the fruit had the least variation with values ≈8.3 g kg^{-1}, whereas that of leaves varied from 21.0 to 25.5 g kg^{-1}.

Nitrogen recovery from the labeled N source was greater for AN (39.5%) than for UR (25.5%) (Table 3). Recovery may have been slightly underestimated since roots were not totally collected from soil and there was also probably considerable loss of N due to senescence and shedding of mature leaves, flowers, and young fruit. Feigenbaum et al. (1987) reported that ^{15}N-uptake efficiency from labeled KNO_3 for 22-yr-old Shamouti orange trees was 40%. In their study, labeled fertilizer was applied with irrigation water in five monthly applications from April to August. Boaretto et al. (1999) found 33% ^{15}N recovery from UR applied to soil for 1-yr-old 'Pêra' orange trees cultivated in closed pots where leaching losses of NO_3 were prevented.

Most of the total ^{15}N recovery occurred in the AG portion of the tree and accounted for 32.8 and 18.9% of applied N for AN and UR treatments, respectively (Table 3). In contrast, Legaz et al. (1982) reported 50 to 60% of total tree ^{15}N recovery in the AG portion of 5-yr-old calamondin trees [*C. mitis Blanco* (= *C. madurensis Lour.*)] grown in pots filled with siliceous sand and irrigated with a modified Arnon and Hoagland nutrient solution. In our experiment, the total N concentration of fruit was similar between AN and UR treatments and the values of Ndff were significantly different ($P < 0.01$) (Fig. 4A and 4B). Plants treated with AN showed Ndff values of 35.2, 25.7, 19.8, and 16.6% from April to December, whereas for the UR treatment, the respective values were 18.5, 14.6, 9.3, and 9.2% (Fig. 4A). This suggests that fruit development was dependent on N from reserve organs at least during early growth. Fruit was responsible for the highest recovery of applied ^{15}N, followed by leaves, twigs, and trunk and then root biomass (Table 3). Experiments with ^{15}N labeled fertilizers have shown that rate of N uptake by citrus trees is affected by immediate growth of trees with fruit as the dominant sink for N (Dasberg, 1987; Lea-Cox et al., 2001). Average concentrations of fruit total N in our study were 16.3 g kg^{-1} on 19 April, 10.7 g kg^{-1} on 8 June, 8.8 g kg^{-1} on 9 September, and 8.4 g kg^{-1} on 1 Dec. (Fig. 4B). Legaz and Primo-Millo (1988) showed that fruit was the only organ of 4-yr-old Valencia orange trees that accumulated N from the fertilizer and from the reserve N stored in other organs; even though the total N concentration of fruit changed as follows: 22.5 g N kg^{-1} during fruit set, 17.2 during second flush of leaves, and 13.6 at dormancy. Paramasivam et al. (2000) observed that N concentrations of Hamlin orange fruit decreased with their enlargement during June through November.

The ^{15}N recovery in the fruit was 10.2 to 18.5% (Table 3), which represents the N absorbed from the labeled fertilizer applied in February. Apparent low recovery of added N by fruit is also reported for other fruit crops. Field measurements on mature almond trees showed that 10 to 25% of ^{15}N was recovered in the harvested fruit (Weinbaum et al., 1984). The removal of ^{15}N in harvested kiwi fruit in the first year after N fertilization was ≈5 to 6% of the applied labeled fertilizer, even though total removal by the vine tree was ≈50% (Ledgard and Smith, 1992). Weinbaum and Van Kessel (1998) found that total almond tree recovery of applied ^{15}N-depleted N fertilizer during a 6-yr experimental period

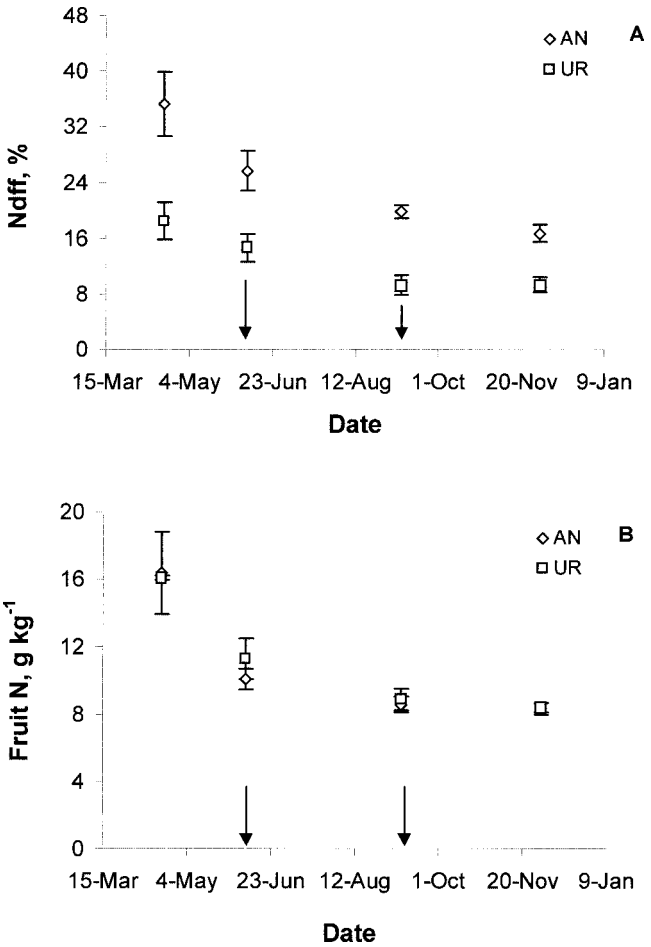

Fig. 4. Percentage of N derived from the labeled fertilizer (%Ndff) applied in the spring (15 February) in 'Hamlin' orange fruit and total N concentration of fruit during the course of the experiment. AN = NH_4NO_3 treated trees, UR = urea treated trees. Vertical bars are the standard error of the mean (n = 3). Arrows indicate further application of nonlabeled fertilizer. (A) Nitrogen in the fruit derived from the labeled fertilizer vs. time; (B) N concentration in the fruit vs. time.

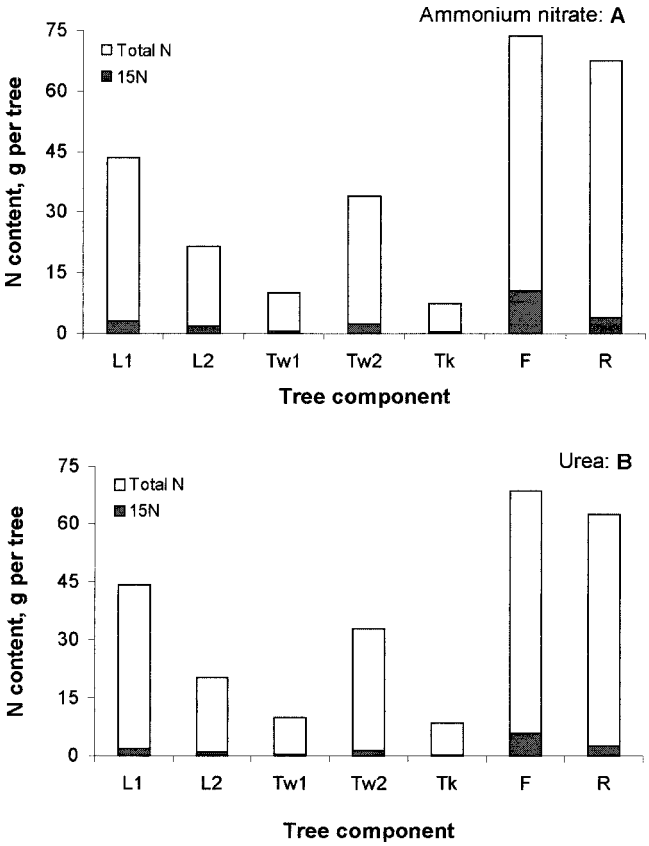

Fig. 5. Total N and ^{15}N contents of tree components 280 d after application of labeled N as ammonium nitrate or urea. L1 = summer plus fall 1999 leaf flush; L2 = spring 1999 plus older leaf flush; Tw1 = twigs > 1.5 cm; Tw2 = twigs ≤ 1.5 cm; Tk = trunk; F = fruit; and R = total roots within the 45-cm soil depth layer. (A) NH_4NO_3 treated trees; (B) urea-treated trees.

was 29.4%. Fruits were the dominant sink, and accounted for 78% of the labeled fertilizer-N recovered by the trees across the period of study. Percentage recovery of ^{15}N applied to 4-yr-old grapefruit trees decreased with increasing N rate, which varied from 63.1 to 23.5%, respectively, and demonstrated decreased N use efficiency (Lea-Cox et al., 2001).

The fate of added ^{15}N during the spring in different tree components is shown in Fig. 5. The largest amount of ^{15}N was found in fruit (5.8 and 10.5 g tree^{-1}), followed by roots and leaves. The amounts found in woody tissues (i.e., trunk, twigs > 1.5-cm diam., and woody roots) were very low (<0.6 g tree^{-1}). The distribution of labeled N related to the total N content of trees treated with AN was calculated with data presented in Fig. 5. Percentage distribution (g ^{15}N 100 g^{-1} N) was 16.7 for fruit, <8.9 for leaves, <8.6 for twigs, 4.9 for trunk, and 5.9 for roots. Trees that received UR showed percentage distribution (g ^{15}N 100 g^{-1} N) as follows: 9.3 for fruit, <5.5 for leaves, <4.8 for twigs, 2.6 for trunk, and 4.4 for roots.

Data presented are in agreement with those of Feigenbaum et al. (1987), who reported that the highest percentage of fertilizer-^{15}N was found in the new organs (fruit, twigs, and leaves) formed during the previous season for a 22-yr-old Shamouti orange tree. The greatest amount of total N of trees was found in the roots (63.6 and 59.9 g for AN- and UR-treated trees, respectively) what represented ≈37% of N stored in the framework components (leaves, twigs, trunk, taproot, and roots) (Fig. 5) and an important source of reduced N for growth and fruit yield.

CONCLUSIONS

Aboveground biomass of Hamlin orange trees grown on sandy soil with continuously monitored low volume irrigation, expressed as a percentage of the total biomass, was >70%. Fruits were the major component of tree biomass (≈30%). Fibrous roots were concentrated in the 0- to 15-cm depth, and represented >70% of the total BG class within the 0- to 45-cm layer. Recoveries of ^{15}N by citrus trees fertilized during the spring with AN and UR were 39.5 and 25.5%, respectively. This difference in total ^{15}N recovery was attributed to losses of N by NH_3 volatilization, since fertilizers were applied to the soil surface with an alkaline reaction (pH > 7).

Fruit appeared to be a large sink for applied ^{15}N (recovery of 10–18%) and redistributed N in the citrus tree. The average ^{15}N content of the tree biomass was small (8% of total N). The maximum observed quantities were associated with fruit. About 10.5 and 5.8 g ^{15}N tree^{-1} were observed for AN and UR treated trees, respectively. Since fruit represented a large sink of applied ^{15}N as UR and AN, respectively, we confirmed the importance of the spring application of N during early development of fruit.

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