Net Carbon Dioxide Assimilation, Carbon Isotope Discrimination, Growth, and Water-use Efficiency of Citrus Trees in Response to Nitrogen Status

J.P. Syvertsen and M.L. Smith

University of Florida, Institute of Food and Agricultural Sciences, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

J. Lloyd and G.D. Farquhar

Environmental Biology, Research School of Biological Sciences, GPO Box 475, Australian National University, Canberra, ACT 2601, Australia

ADDITIONAL INDEX WORDS. $\delta^{13}\text{C}$, stomatal conductance, evapotranspiration, canopy volume, fruit yield, 'Redblush' grapefruit, *Citrus paradisi*, 'Volkamer' lemon, *C. volkameriana*, sour orange, *C. aurantium*

ABSTRACT. Five- to six-year-old 'Redblush' grapefruit (*Citrus paradisi* Macf.) trees on 'Volkamer' lemon [VL = *C. volkameriana* (Ten. & Pasq.)] or sour orange (SO = *C. aurantium* L.) rootstock, were grown individually in 7.9-m² lysimeters for 2.5 years using low to high rates of fertilizer N. Net CO_2 assimilation (A_{CO_2}) of leaves and leaf dry mass per area (DM/a) increased with leaf N concentration, whereas leaf tissue C isotope discrimination (Δ) decreased. Leaf tissue Δ was negatively related to A_{CO_2} and DM/a. Transient effects of rootstock on leaf N were reflected by similar effects on Δ . There was no effect of leaf N on water-use efficiency (WUE) of leaves ($\text{WUE}_L = A_{\text{CO}_2}/\text{transpiration}$); WUE_L was not correlated with Δ . Although photosynthetic N use efficiency (A_{CO_2}/N) consistently decreased with increased leaf N, Δ was not consistently related to A_{CO_2}/N . Annual canopy growth, tree evapotranspiration (ET), and fruit yield increased with whole tree N uptake. Leaf tissue Δ was negatively related to all of these tree measurements at the end of the second year. By that time, whole-tree WUE (WUE_T , annual canopy growth per ET) also was negatively related to Δ . Larger trees on VL had higher ET than trees on SO, but there were no rootstock effects on WUE_T or on Δ . Leaf tissue Δ was consistently higher than Δ values of trunk and woody root tissues. Citrus leaf tissue Δ can be a useful indicator of leaf N, characteristics of leaf gas exchange, tree growth, yield, and WUE_L in response to N availability.

Evergreen trees can assimilate CO_2 during the year whenever environmental conditions are favorable (Waring, 1991). Since *Citrus* leaves can live from 1 (Kriedemann and Barrs, 1981) to 3 years (Syvertsen et al., 1981), trees can store large amounts of N in their leaves (Kato, 1986). Routine fertilization practices have been developed for citrus to supply sufficient N to avoid deficiencies (Tucker et al., 1995). With increased problems of nitrate pollution (Syvertsen and Smith, 1996), however, citrus growers have been encouraged to reduce N fertilization rates, thereby increasing the risk of N limitations on growth and yield. Low N supply can limit net CO_2 assimilation (A_{CO_2} ; Syvertsen, 1987) and leaf development (Romero-Aranda and Syvertsen, 1996), but, because of stored N, meaningful studies on effects of N limitations on tree growth and yield must be conducted for >1 year. Physiological measurements that integrate tree response to N status and correlate with yield would be invaluable in such studies.

During A_{CO_2} , leaves exhibit discrimination (Δ) against the heavy isotope of ^{13}C in atmospheric CO_2 such that the C in plant tissues becomes depleted in ^{13}C relative to CO_2 in air (Farquhar et al., 1982). The isotopic composition of plant tissue can be measured using mass spectrometry (Ehleringer et al., 1993). Leaf tissue Δ has a diffusive and a biochemical component and is proportional to the ratio of chloroplastic to ambient partial pressure of CO_2 (p_c/p_a ; Farquhar et al., 1989). Factors that limit A_{CO_2} directly, like low N concentration in leaves, increase p_c/p_a and increase Δ . Factors that limit stomatal conductance (g), however,

decrease p_c/p_a and Δ . Since leaf water-use efficiency (WUE_L , ratio of $A_{\text{CO}_2}/\text{transpiration}$) also can be related to p_c/p_a (Ehleringer and Osmond, 1991), Δ can be negatively related to WUE_L (Le Roux et al., 1996; Meinzer et al., 1993). At a given atmospheric humidity, Δ also can be negatively related to the long-term WUE defined as growth or productivity per unit of water used (Johnson and Tieszen, 1993). Leaf tissue Δ has been widely used to evaluate genetic and environmental effects on plant growth (Ehleringer, 1993; Le Roux et al., 1996), leaf nutrient levels (Bender and Berge, 1979; Masle et al., 1992), and photosynthetic N use efficiency ($A_{\text{CO}_2}/\text{leaf N}$, Gutierrez and Meinzer, 1994).

Variations in leaf tissue Δ have been attributed to changes in root length density (White et al., 1990) and in root growth responses to compacted soil (Masle and Farquhar, 1988). Horticultural fruit trees provide an interesting system in which to study the relative importance of roots and shoots in determining tree growth and physiological responses since the same fruit variety can be grown as the scion on genetically different rootstocks. Rootstocks influence tree growth, fruit quality, and yield (Castle et al., 1993), partly by altering plant water relations, mineral nutrition (Syvertsen and Graham, 1985), and A_{CO_2} (Syvertsen et al., 1988). Leaf tissue Δ has been negatively related to water use characteristic of peach rootstocks (Bongi et al., 1994) and to leaf N concentrations in citrus trees on different rootstocks (Syvertsen and Lloyd, 1994). Although leaf tissue Δ should reflect physiological responses and characteristics of tree growth, the relationship between leaf and root tissue Δ of grafted fruit trees has not been investigated. Our purpose was to contrast the effects of N status on net gas exchange of leaves, tree growth, water use, and Δ of citrus trees grafted onto a fast- or slow-growing rootstock. We hypothesized that Δ of leaf tissues could be correlated to short-term net gas exchange of leaves and also to long-term tree growth, N uptake, fruit yield, and WUE_L .

Received for publication 18 Mar. 1996. Accepted for publication 30 Aug. 1996. Florida Agricultural Experiment Station journal series no. R-05068. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

Fig. 1. The effect of leaf N concentration on (a) net assimilation of CO_2 (A_{CO_2}), (b) leaf dry mass per area (DM/a), (c) leaf tissue carbon isotope discrimination (Δ), and (d) leaf nitrogen use efficiency (A_{CO_2}/N) of 'Redblush' grapefruit leaves on 'Volkamer' lemon (open symbols) or sour orange rootstocks (closed symbols) during Spring 1992 (□, ●), Spring 1993 (◇, ◆), and Winter 1993 (○, ●) measurement periods. Each symbol represents the mean of three leaves on each of 18 trees. Correlation coefficients (r) are associated with each line; critical r for $n - 2 = 16$ at $P < 0.05 = 0.461$.

surfaces were maintained weed free with herbicide (glyphosate) and hand cultivation so that no leaf litter accumulated. Trees were sprayed periodically for pests. All presented data are from years 2 and 3 (1992, 1993) when trees were 5 to 6 years old.

All trees were similarly well-irrigated at ≈ 0.6 of class A pan evaporation using $60 \text{ L} \cdot \text{h}^{-1}$ microsprinklers that wet most of the soil surface area under each tree with a wetted diameter of $\approx 3 \text{ m}$. Total annual rainfall was 1395 and 1105 mm during 1992 and 1993, respectively, compared to a mean annual rainfall of $\approx 1250 \text{ mm}$ in central Florida. Total irrigation water applied during 1992 and 1993 averaged ≈ 347 and $506 \text{ mm} \cdot \text{year}^{-1}$ (Syvertsen and Smith, 1996).

Materials and Methods

TREE CULTURE. We used 18 trees that were part of a 2.5-year (1991–93) study to evaluate the effect of low, intermediate, and high rates of fertilizer N on growth, N uptake, and leaching losses from lysimeter-grown citrus trees (Syvertsen and Smith, 1995, 1996). The study was conducted in subtropical central Florida using native Candler fine sand soil having a pH of ≈ 5.5 and $< 0.8\%$ organic matter (Alva and Syvertsen, 1991). Each lysimeter tank was $\approx 3.4 \text{ m}$ in diameter, 0.9 m deep, and contained $\approx 7.9 \text{ m}^3$ of soil (Boman and Syvertsen, 1991). Nine 4-year-old 'Redblush' grapefruit (*Citrus paradisi* Macf.) trees on 'Volkamer' lemon [VL = *C. volkameriana* (Ten. & Pasq.)] rootstock and nine similar trees on sour orange (SO = *C. aurantium* L.) rootstock were randomly transplanted into 18 single-tree drainage lysimeter tanks during March 1991. Trees were well-nourished before transplantation and were fertilized uniformly at $\approx 180 \text{ g N/tree}$ per year for 3 months until the N rate treatments (described below) were begun in July 1991. Lemon rootstocks produce relatively fast-growing (vigorous) trees (Castle et al., 1993), whereas trees on SO are slower growing and generally have leaves with relatively low N concentrations (Reuther, 1973). Lysimeter tanks were arranged in two rows with a spacing of 4.9 m within the row and 6.1 m between rows so that tree canopies did not touch. During the study, soil

FERTILIZER TREATMENTS. Three replicate trees on each rootstock were randomly assigned one of three (low, intermediate, or high) N levels. Soluble N, P, K, and Mg (plus microelements) were applied about once per week by injecting (in %) 2–2–8–0.5, 6–2–8–0.5, or 12–2–8–0.5 liquid fertilizer into the irrigation system. We fertilized weekly during the year but avoided rainy periods to minimize nutrient leaching. About 20 milliliters of water was sampled from the microsprinklers during every fertilizer application and used to calculate the amount of nitrogen applied. The N source was NH_4NO_3 . The three application rates varied slightly from targeted rates, but actual rates averaged ≈ 80 , 180 , and 540 g N/tree per year during the end of 1991 and 1992. At the beginning of 1993, rates were increased to ≈ 125 , 450 , and 860 g N/tree per year (Syvertsen and Smith, 1996). The recommended rate for 5- to 6-year-old trees is $\approx 550 \text{ g N/tree}$ per year (Tucker et al., 1995).

NET GAS EXCHANGE MEASUREMENTS AND Δ . Net A_{CO_2} and transpiration (E) of single leaves were measured, and g calculated using a portable photosynthesis system (LI-6200; LI-COR, Lincoln, Neb.) and 1-L cuvette. Leaf WUE (WUE_L) was calculated as A_{CO_2}/E . All measurements were made on fully expanded mature leaves that were healthy in appearance and located in sun-exposed, southeastern canopy positions. Gas exchange measurements were made during three sampling periods that were selected for their similar clear, cool weather. During Spring (February–March)

Table 1. The effect of rootstock (RS, VL = Volkamer lemon; SO = Sour orange) on mean (SE) values of leaf characteristics of leaves on 'Redblush' grapefruit trees ($n = 9$) in Spring 1992.^a

	Leaf N ($\text{mmol} \cdot \text{m}^{-2}$)	A_{CO_2} ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	E ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	WUE_L ($\mu\text{mol} \cdot \text{mmol}^{-1}$)	DM/a ($\text{g} \cdot \text{m}^{-2}$)	A_{CO_2}/N ($\mu\text{mol} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$)	Δ ($^\circ/\text{‰}$)
RS							
VL	230.6 (19.6)*	8.02 (0.35)*	2.36 (0.16) ^{NS}	3.57 (0.29) ^{NS}	154.2 (5.8)*	37.0 (3.8) ^{NS}	20.07 (0.13)*
SO	289.3 (24.4)	9.67 (0.45)	2.41 (0.19)	4.19 (0.35)	167.4 (5.0)	34.4 (1.9)	19.24 (0.15)

^aAbbreviations: A_{CO_2} = assimilation of CO_2 ; E = transpiration; WUE_L = leaf water-use efficiency; DM/a = leaf dry mass per area; A_{CO_2}/N = A_{CO_2} per leaf N; Δ = ^{13}C discrimination.

^{NS}, Nonsignificant or significant rootstock effect at $P < 0.05$.

Table 2. Correlation coefficients between leaf tissue C isotope discrimination (Δ) and leaf characteristics of leaves on 'Redblush' grapefruit trees (n=18) during Spring 1992 and 1993 along with Winter 1993 measurement periods.^z

	Δ (‰)	A_{CO_2} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	E ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	WUE_L ($\mu\text{mol}\cdot\text{mmol}^{-1}$)	DM/a ($\text{g}\cdot\text{m}^{-2}$)	A_{CO_2}/N ($\mu\text{mol}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$)
Spring 1992						
A_{CO_2}	-0.74	1	-0.02	0.55	0.50	0.08
E	-0.14		1	-0.82	0.29	-0.35
WUE_L	-0.33			1	0.01	0.33
DM/a	-0.64				1	-0.74
A_{CO_2}/N	0.31					1
Spring 1993						
A_{CO_2}	0.16	1	0.66	-0.18	0.32	0.39
E	0.08		1	-0.82	0.18	0.06
WUE_L	0.04			1	0.03	0.16
DM/a	-0.73				1	-0.53
A_{CO_2}/N	0.70					1
Winter 1993						
A_{CO_2}	-0.46	1	0.58	0.45	0.24	0.51
E	-0.50		1	-0.45	0.07	0.04
WUE_L	-0.01			1	0.13	0.53
DM/a	-0.48				1	0.18
A_{CO_2}/N	-0.33					1

^zAbbreviations: A_{CO_2} = assimilation of CO_2 ; E = transpiration; WUE_L = leaf water-use efficiency; DM/a = leaf dry mass per area; A_{CO_2}/N = A_{CO_2} per leaf N; Δ = ^{13}C discrimination. Critical r for $n = 16$ at $P < 0.05 = 0.461$.

1992 and 1993, leaves were ≈ 5 to 6 months old. Leaves measured in Winter (November–December) 1993 were ≈ 7 to 8 months old. Within each sampling period, three tagged leaves on each tree were measured repeatedly on 3 separate days. Measurements were done in the morning (0900 to 1100 hr) to avoid high temperatures and vapor-pressure deficits (VPDs) in the measurement cuvette. During all measurements, leaf temperatures were 21 to 28 °C, photosynthetic photon flux (PPF) was $>900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and leaf-to-air VPD was 1.2 to 3.2 kPa. A few measurements that were recorded at temperatures >28 °C and at a VPD >3.2 kPa, had a $g < 50 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and were eliminated from the data set. The three repeated measurements (occasionally only two) from each measured leaf were averaged to determine mean values for each leaf. An average A_{CO_2} value for each tree was calculated as the mean of the three leaves on each tree. At the end of each sampling period, each measured leaf was harvested and its leaf area was measured with a leaf area meter (LI-3000), surface washed with deionized water, dried at 60 °C for at least 48 h, and ground to a powder for Kjeldahl analysis of total N. An average leaf N concentration for each tree was calculated as the mean of the three leaves on each tree.

Leaf tissue ^{13}C composition of each measurement leaf was measured using a ratio mass spectrometer (VG SIRA/24) and used to calculate the ratio of ^{13}C to ^{12}C [δ_p per mil (‰) relative to PeeDee Belemite; Farquhar et al., 1989]. The isotopic composition of CO_2 in air (δ_a) was assumed to be -7.8 ‰ (Francey et al., 1995) and, along with δ_p , was used to calculate discrimination against ^{13}C (Δ) as

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$$

(Farquhar et al., 1982; 1989). Thus, Δ is opposite in sign to δ (or $\delta^{13}C$), which has been used by others to describe C isotope discrimination in plants (e.g., Le Roux et al., 1996), but Δ is the preferred term used to describe isotope effects (Farquhar et al., 1989).

An average leaf tissue Δ for each tree was calculated as the mean Δ of the three leaves per tree used for gas exchange and N

measurements. We also averaged Δ of three leaves that were ≈ 7 to 8 months old from each tree in December 1992 when gas exchange was not measured. We sampled woody tissues from each tree to determine potential variations in woody tissue Δ that could be attributed to either scion or rootstock. In December 1992, we sampled ≈ 5 g dry mass of bark tissue from each tree trunk at a height of 30 cm and also from bark of woody roots just below the soil surface for analysis of tissue Δ . Any surface contamination on the exterior of bark tissues was scraped away with a knife. In December 1993, ≈ 5 g dry mass of trunk wood and root wood also were sampled from inside the bark tissues of each tree sampled as in the previous year. Woody tissue samples were dried and ground to a powder, and their Δ values were measured as the leaf samples.

Table 3. Mean (SE) carbon isotope discrimination Δ (‰) of tissues from 'Redblush' grapefruit trees (n = 9) on Volkamer lemon (VL) or Sour orange (SO) rootstocks (RS) sampled in December 1992 and 1993.

RS	Tissue	Δ (‰)	
		1992	1993
VL	Leaf	20.32 (0.09)	19.98 (0.21)
	Trunk bark	19.80 (0.10)	18.66 (0.24)
	Root bark	17.70 (0.23)	17.72 (0.14)
	Trunk wood	---	18.20 (0.24)
	Root wood	---	17.52 (0.24)
SO	Leaf	20.21 (0.10)	19.82 (0.19)
	Trunk bark	19.70 (0.14)	18.02 (0.13)
	Root bark	17.73 (0.15)	17.58 (0.12)
	Trunk wood	---	17.71 (0.33)
	Root wood	---	17.35 (0.27)
RS		NS	**
Tissue		***	***
RS $\times$ tissue		NS	NS

^zWood samples were not evaluated in 1992.

NS, **, *** Nonsignificant or significant at $P < 0.01$ or 0.001.

Fig. 2. The relationship of annual nitrogen uptake with (a) canopy volume, (b) evapotranspiration (ET), (c) fruit yield, and (d) leaf tissue carbon isotope discrimination (Δ) of 'Redblush' grapefruit trees in December 1992 ($\square$ — $\blacksquare$) and December 1993 ($\circ$ — $\bullet$). Each symbol represents an individual tree; open symbols are for trees on 'Volkamer' lemon and closed symbols are for trees on sour orange rootstocks. Correlation coefficients (r) are associated with each line; critical r for $n - 2 = 16$ at $P < 0.05 = 0.461$.

ignored the relatively small amount of N retained in the sandy soil (Alva and Syvertsen, 1991). Only trace amounts of NH_4 were occasionally collected in the drainage water, so NH_4 was added to NO_3 data and expressed as total N (Syvertsen and Smith, 1996).

DATA ANALYSES. Relationships between variables were tested for significance using linear correlation analyses (GLM, SAS Institute, Cary, N.C.). Critical correlation coefficients (r) for $n = 16$ ($18 - 2$) at $P < 0.05 = 0.461$. Analysis of variance using rootstock (RS) as a fixed categorical variable was used to test for significant effects of RS. Comparisons of Δ of tree tissues were tested for significant differences using a 2 RS $\times$ 3 (or 5) tissues factorial analysis of variance.

Results

CANOPY GROWTH, YIELD, WUE, AND N UPTAKE. To evaluate tree growth, tree canopy dimensions were measured in January 1992, December 1992, and December 1993 to calculate canopy volume (m^3) as a modified prolate spheroid (Albrigo et al., 1975). Canopy growth was calculated as the annual difference in canopy volume. Vegetative biomass was not measured. Fruit yield (FY, kg/tree) was determined at harvest in December 1992 and 1993. Evapotranspiration (ET) values for each tank were calculated as the water used between saturating rains from differences between water input (rainfall plus irrigations) and water collected in the drains. This allowed us to ignore soil water storage. Annual ET was the total water used (m) during the year based on the 8.8-m^2 surface area of each lysimeter. Annual whole-tree WUE (WUE_T) was calculated as canopy growth/ET ($\text{m}^3 \cdot \text{m}^{-1}$). The annual amount (g) of nitrogen taken up by each tree (N_{up}) was calculated from the annual amount of N applied, minus the total N recovered in the drainage water, minus the unaccounted for N from a lysimeter tank with no tree (Syvertsen and Smith, 1996). This estimate of N_{up}

LEAF N, NET GAS EXCHANGE AND LEAF TISSUE Δ . Net A_{CO_2} and leaf DM/a were positively correlated with leaf N in all three measurement periods (Fig. 1 a and b). High N trees on SO initially had higher leaf N concentration, higher A_{CO_2} , greater DM/a, and lower Δ than those on VL in Spring 1992 (Table 1), but there was no effect of rootstock on any of these variables in 1993 (data analysis not shown). Leaves on SO also had lower Δ than those on VL in 1992, but, again, there was no effect of rootstock on Δ thereafter. There was no effect of rootstock on leaf transpiration (E), WUE_L , or A_{CO_2}/N . Leaf tissue Δ was negatively related to leaf N (Fig. 1c). Overall, A_{CO_2} did not increase proportionally with leaf N as shown by the consistent decrease in A_{CO_2}/N with increased leaf N (Fig. 1d). Leaf Δ was negatively related with A_{CO_2}/N only in Spring 1993 (Table 2). Leaf Δ was consistently negatively related to DM/a in all three sampling periods. Leaves with high DM/a had low A_{CO_2}/N except in Winter 1993 when leaf N and DM/a were relatively low. There were negative correlations between Δ and A_{CO_2} in the first and third

Table 4. The effect of rootstock (RS, VL = Volkamer lemon; SO = Sour orange) on mean (SE) tree characteristics of 'Redblush' grapefruit trees ($n = 9$) in December 1992 and 1993.^z

RS	N_{up} ($\text{g} \cdot \text{year}^{-1}$)	V_c (m^3)	FY (kg)	ET (m)	WUE_T ($\text{m}^3 \cdot \text{m}^{-1}$)
1992					
VL	221.3 (57.6)*	5.3 (0.5)*	44.1 (5.4)*	1.8 (0.3)*	1.8 (0.1) ^{NS}
SO	161.2 (37.1)	4.3 (0.4)	11.7 (2.4)	1.6 (0.1)	2.1 (0.2)
1993					
VL	247.1 (63.6)*	9.5 (0.9)*	61.3 (14.3) ^{NS}	1.6 (0.1)*	2.6 (0.2) ^{NS}
SO	168.5 (38.5)	7.5 (0.7)	62.6 (5.9)	1.3 (0.1)	2.5 (0.3)

^zAbbreviations: N_{up} = nitrogen uptake; V_c = canopy volume; FY = fruit yield; ET = evapotranspiration; WUE_T = tree water-use efficiency.

^{NS}, *Nonsignificant or significant rootstock effect at $P < 0.05$.

Table 5. Correlation coefficients between leaf tissue C isotope discrimination and tree characteristics of 'Redblush' grapefruit trees (n = 18) in December 1992 and 1993.²

	Δ (‰)	ET (m)	FY (kg)	Leaf N (mmol·m ⁻²)
1992				
V _c (m ³)	0.29	0.91	0.34	0.29
ET	0.03	1	0.53	0.40
FY	0.13		1	0.25
Leaf N	0.21			1
N _{up} (g·year ⁻¹)	0.14 ³			0.65
1993				
V _c	-0.64	0.91	0.57	0.74
ET	-0.44	1	0.74	0.20
FY	-0.55		1	0.47
Leaf N	-0.82 ³			1
N _{up} (g·year ⁻¹)	-0.62 ³			0.43

²Abbreviations: Δ = ¹³C discrimination, V_c = canopy volume; ET = evapotranspiration; FY = fruit yield; N_{up} = nitrogen uptake.

³Correlation between Δ and leaf N in 1993 appears in Fig. 1c; correlations between Δ and N_{up} are shown in Fig. 2d. Critical *r* for n = 16 at *P* < 0.05 = 0.461.

sampling periods but not in Spring 1993. Net A_{CO2} and *g* were strongly related in all three measurement periods (*r* > 0.70), but correlations between A_{CO2} and *E* and between A_{CO2} and WUE_L were inconsistent (Table 2). There were no relationships between WUE_L and Δ . Leaf N affected A_{CO2} and *E* in a similar manner as there was no relationship between leaf N and WUE_L (data not shown).

LEAF, BARK, AND WOOD TISSUE Δ . In both years, leaf tissue Δ was higher than that of bark and wood tissues (Table 3). There were no significant effects of rootstock on Δ in 1992 but, in 1993, VL had higher Δ than SO when compared across all tissues. Woody tissue Δ ranked similarly within each rootstock in both years.

CANOPY GROWTH, ET, YIELD, AND N UPTAKE. During both years, larger trees on VL took up more N and used more water than smaller trees on SO (Fig. 2, Table 4). Older, larger trees in 1993 actually had lower ET than in 1992 (Fig. 2b), reflecting the greater evaporation from soil due to greater evaporative demands in 1992 (Syvertsen and Smith, 1996). Fruit yields (FY) also were positively correlated to N_{up} in both years but only significantly so in 1993 (Fig. 2c). Fruit yields from trees on VL were greater than from trees on SO in 1992 but not in 1993 (Table 4) when low N trees on VL had very low FY. Annual N uptake was not related to Δ in 1992 but trees with the highest N uptake had the lowest discrimination in 1993 (Fig. 2d).

In 1992, there was not yet a relationship between Δ and canopy volume, ET or fruit yield, but, in 1993, Δ was negatively related to all of these variables (Table 5). Yield was positively related to ET in both years, and largest trees yielded the most in 1993. Leaf N concentration in December was positively related to N_{up} in both years. By 1993, trees with the largest canopies also had highest leaf N concentrations.

WUE AND Δ . Although there was no correlation between WUE_T and Δ in 1992, Δ was negatively related to WUE_T by the end of 1993 (Fig. 3). The close relationships between canopy volume, fruit yield, and ET resulted in remarkably similar correlations between Δ and FY/ET (data not shown) as between Δ and WUE_T. Larger trees had higher WUE_T as WUE_T increased with canopy volume in both years (*r* = 0.79, 0.49). There were no effects of rootstock on WUE_T or Δ (Table 3).

Discussion

At the beginning of this study, all 4-year-old trees were similar in size and had similar leaf N concentrations regardless of rootstock. After N treatments had been in place for ≈9 months, by Spring 1992, faster-growing trees on VL were already larger but rates of N uptake apparently were not adequate for leaf N levels to be correlated with the rapidly expanding canopy volumes. Relatively small trees on SO initially had higher leaf N concentrations, higher A_{CO2}, and lower Δ than the larger trees on VL. This was a transient effect, however, because, by the end of 1992, canopy volume and total N uptake were closely related and both were related to leaf N in 1993. By Spring 1993, there were no rootstock effects on leaf N, net gas exchange, or on leaf tissue Δ . Thus, leaf Δ reflected changing trends in leaf N and A_{CO2} that could be attributed to rootstock effects that diminished as trees grew in response to N treatments.

Although canopy size, ET, and fruit yield were closely related in both years, Δ was correlated with these variables only in the second year. In addition, Δ was only related to WUE_T in 1993. It is possible that the large soil evaporation component of ET of the relatively small trees contributed to the lack of correlation between WUE_T and Δ in 1992. More importantly, however, the mature leaves sampled at the end of each year expanded during the previous spring. Thus, leaf tissue Δ in 1992 represented leaves that expanded during a period only 9 months after the N treatments had begun. Trees probably had not yet responded to these recent established treatments. At the end of 1993, Δ was negatively related to leaf N and N_{up} (Table 5). By that time, larger trees had lower Δ , a correlation described by Zhang et al. (1994) for deciduous conifers, and there was a negative relationship between Δ and WUE_T. This underscores the importance of sample timing, especially of long-lived evergreen leaves, since leaf tissue Δ probably reflected isotope effects on C committed to leaf structure that occurred during the leaf expansion period.

The lack of correlation between short-term WUE_L and Δ (Table 2) is in contrast to the negative relationship between Δ and long-term WUE_T in 1993. Even using average gas exchange data from three leaves from each tree, Δ was not related to WUE_L. This may have been related to the variability in measurements of A_{CO2} since A_{CO2} was inconsistently related to Δ over the three measurement periods. Even the strongest correlation between A_{CO2} and Δ (*r* = -0.74, Table 2), however, indicated that only 55% (*r*²) of the variation in Δ could be accounted for by variations in A_{CO2}. Although A_{CO2} and *g* were strongly correlated, variations in VPD during gas exchange measurements may have contributed to poor correlations between *E* and A_{CO2}, thus precluding any relationship between WUE_L and Δ . Drought stress can complicate relationships

between WUE_L and Δ (Le Roux et al., 1996), but strong correla-

Fig. 3. The relationship between leaf tissue C isotope discrimination (Δ) and whole-tree water-use efficiency (WUE_T) in December 1992 (□) or December 1993 (○). Symbols are as in Fig. 2. Correlation coefficients (*r*) are associated with each line; critical *r* for n = 16 at *P* < 0.05 = 0.461.

tions between short- and long-term WUE with Δ have been described for irrigated and drought-stressed larch trees (Zhang and Marshall, 1994). In that case, too, correlations between Δ and long-term WUE_T were stronger than those between Δ and WUE_L.

Leaf N levels were lowest in the winter at the end of the study along with low leaf DM/a and relatively high Δ . Within each sampling period, thicker leaves with a high DM/a, had higher N, higher A_{CO₂}, and lower Δ than leaves with relatively low DM/a. A similarly consistent relationship between Δ and leaf mass per area has been reported for peanut leaves regardless of environmental conditions (Rao and Wright, 1994). There were consistent negative relationships, however, between A_{CO₂}/N and leaf N. The decrease in A_{CO₂}/N with increased leaf N is a phenomenon that we have observed in *Citrus* grown at different light intensities (Lloyd et al., 1992) and fertilized with different N rates (Lea-Cox and Syvertsen, 1996; Romero-Aranda and Syvertsen, 1996). Similar to other evergreen leaves (Waring, 1991), citrus leaves can accumulate high N concentrations that do not necessarily translate into higher photosynthetic activities. The variability in measurements of A_{CO₂} probably contributed to the weak correlations between leaf tissue Δ and A_{CO₂}/N, which were not as consistent as similar relationships discussed by Gutierrez and Meinzer (1994).

Consistently greater Δ values in leaves than in woody tissues can be at least partially attributed to the larger lipid content in leaves, since lipids are depleted in ¹³C relative to other plant tissues (Farquhar et al., 1989). Similar patterns between Δ of leaf and wood tissues have been observed in other species (Leavitt and Long, 1992; Medina et al., 1986) and could implicate differences in respiration or slower changes in isotopic composition as C becomes committed to cellulose (Medina et al., 1991). There were no rootstock species difference in Δ in 1992, but there was a significant rootstock effect in 1993 (Table 3). This was due to the consistently higher Δ of VL than that of the same SO tissues, but such small differences probably were not physiological meaningful. The small standard deviations associated with Δ of replicate trees and the similarity of root bark samples from 1992 to 1993 underscores the repeatability of Δ value.

Although it is difficult to describe tree performance from short-term leaf gas exchange characteristics, leaf tissue Δ integrated leaf responses and so was a good indicator of relative differences in leaf N, A_{CO₂}, and DM/a as influenced by rootstock and N supply. Canopy growth, leaf N concentration, ET, and fruit yield were correlated to whole-tree N uptake. Tree N_{up} and WUE_T were negatively related to Δ by the end of the second year. Based on Δ of leaves that expanded after trees had responded to the N treatments, consistent year-to-year differences in leaf tissue Δ could be used to predict differences in tree growth and fruit yield.

Literature Cited

- Albrigo, L.G., C.A. Anderson, G.J. Edwards, F.W. Bistline, W.J. Hepburn, and T. Cary. 1975. Yield estimates of 'Valencia' orange research plots and groves. *Proc. Fla. State Hort. Soc.* 88:44-49.
- Alva, A.K. and J.P. Syvertsen. 1991. Irrigation water salinity affects soil nutrient distribution, root density and leaf nutrient levels of citrus under drip fertigation. *J. Plant Nutr.* 14:715-727.
- Bender, M.M. and A.J. Berge. 1979. Influence of N and K fertilization and growth temperature on ¹³C/¹²C ratios of Timothy (*Phleum pratense* L.). *Oecologia* 44:117-118.
- Boman, B.J. and J.P. Syvertsen. 1991. Drainage lysimeters for high water table citrus studies, p. 318-325. In: R.G. Allen, T.A. Howell, W.O. Pruitt, I.O. Walter, and M.E. Jensen (eds.). *Lysimeters for evapotranspiration and environmental measurements*. Proc. ASCE Intl. on Lysimetry, ASCE, New York.
- Bongi, G., A. Palliotti, P. Rocchi, and G. Roselli. 1994. Evaluation of water use efficiency in peach grafted on different interspecific hybrid rootstocks. *Plant Physiol. Biochem.* 32:149-157.
- Castle, W.S., D.P.H. Tucker, A.H. Krezdorn, and C.O. Youtsey. 1993. Rootstocks for Florida citrus. 2nd. ed. SP-42. Inst. Food Agr. Sci., Univ. Fla., Gainesville.
- Ehleringer, J.R. 1993. Variation in leaf carbon isotope discrimination in *Encelia farinosa*: Implications for growth, competition and drought survival. *Oecologia* 95:340-346.
- Ehleringer, J.R., A.E. Hall, and G.D. Farquhar (eds.). 1993. *Stable isotopes and plant carbon-water relations*. Academic Press, San Diego.
- Ehleringer, J.R. and C. Barry Osmond. 1991. Stable isotopes, p. 281-300. In: R.W. Pearcy, J. Ehleringer, H.A. Mooney, and P.W. Rundel (eds.). *Plant physiological ecology. Field methods and instrumentation*. Chapman and Hall, London.
- Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and CO₂ concentration in leaves. *Austral. J. Plant Physiol.* 9:121-137.
- Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:503-537.
- Francey, R.J., P.P. Tans, C.E. Allison, I.G. Enting, J.W.C. White, and M. Troller. 1995. Changes in oceanic and terrestrial carbon uptake since 1982. *Nature* 373:326-330.
- Gutierrez, M.V. and F.C. Meinzer. 1994. Carbon isotope discrimination and photosynthetic gas exchange in coffee hedge rows during canopy development. *Austral. J. Plant Physiol.* 21:207-219.
- Johnson, R.C. and L.L. Tieszen. 1993. Carbon isotope discrimination, water relations and gas exchange in temperate grass species and accessions, p. 281-296. In: J.R. Ehleringer, A.E. Hall, and G.D. Farquhar (eds.). *Stable isotopes and plant carbon-water relations*. Academic Press, San Diego.
- Kato, T. 1986. Nitrogen metabolism and utilization in citrus, p. 81-216. In: J. Janick (ed.). *Horticultural reviews*. vol. 8. AVI Publishing Co.
- Kriedemann, P.E. and H.D. Barrs. 1981. Citrus orchards, p. 325-417. In: T.T. Kozlowski (ed.). *Water deficits and plant growth*. vol. 6. Academic Press, New York.
- Lea-Cox, J.D. and J.P. Syvertsen. 1996. How nitrogen supply affects growth and nitrogen uptake, use efficiency, and loss from *Citrus* seedlings. *J. Amer. Soc. Hort. Sci.* 121:105-114.
- Leavitt, S. and A. Long. 1982. Evidence for ¹³C/¹²C fractionation between tree leaves and wood. *Nature* 298:742-744.
- Le Roux, D., W.D. Stock, W.J. Bond, and D. Maphanga. 1996. Dry mass allocation, water use efficiency and δ^{13} C in clones of *Eucalyptus grandis*, *E. grandis* × *camaldulensis* and *E. grandis* × *nitens* grown under two irrigation regimes. *Tree Physiol.* 16:497-502.
- Lloyd, J., J.P. Syvertsen, P.E. Kriedemann, and G.D. Farquhar. 1992. Low conductances for CO₂ diffusion from stomata to the sites of carboxylation in leaves of woody species. *Plant Cell Environ.* 15:873-899.
- Masle, J. and G.D. Farquhar. 1988. Effects of soil strength on the relations of water use efficiency and growth to carbon isotope discrimination in wheat seedlings. *Plant Physiol.* 86:32-38.
- Masle, J., G.D. Farquhar, and S.C. Wong. 1992. Transpiration efficiency and plant mineral nutrient content are related among genotypes of a range of species. *Austral. J. Plant Physiol.* 19:709-721.
- Medina, E., G. Montes, E. Cuevas, and Z. Rokzandic. 1986. Profiles of CO₂ and δ^{13} C values in tropical rain forests of the upper Rio Negro Basin, Venezuela. *J. Trop. Ecol.* 2:207-217.
- Medina, E., L. Sternberg, and E. Cuevas. 1991. Vertical stratification of δ^{13} C values in closed natural and plantation forests in the Luquillo mountains, Puerto Rico. *Oecologia* 87:369-372.
- Meinzer, F.C., G. Goldstein, and D.A. Grantz. 1993. Carbon isotope discrimination and gas exchange in coffee during adjustment to different soil moisture regimes, p. 327-345. In: J.R. Ehleringer, A.E. Hall, and G.D. Farquhar (eds.). *Stable isotopes and plant carbon-water relations*. Academic Press, San Diego.
- Rao, R.C.N. and G.C. Wright. 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. *Crop Sci.* 34:98-103.

- Reuther, W. (ed.). 1973. The citrus industry. vol. 3. Production technology. Univ. Calif. Press, Agr. Sci. Publ., Berkeley. p. 448–474.
- Romero-Aranda, R.M. and J.P. Syvertsen. 1996. The influence of foliar-applied urea N and saline solutions on net gas exchange of citrus leaves. J. Amer. Soc. Hort. Sci. 121:501–506.
- Syvertsen, J.P. 1987. Nitrogen content and CO₂ assimilation characteristics of *Citrus* leaves. HortScience 22:289–291.
- Syvertsen, J.P. and J.H. Graham. 1985. Hydraulic conductivity of roots, mineral nutrition and leaf gas exchange of citrus rootstocks. J. Amer. Soc. Hort. Sci. 110:865–869.
- Syvertsen, J.P. and J. Lloyd. 1994. Citrus, p. 65–99. In: B. Schaffer and P. Andersen (eds.). Handbook of environmental physiology of fruit crops. vol. 2. Sub-tropical and tropical crops. CRC Press, Boca Raton, Fla.
- Syvertsen, J.P. and M.L. Smith. 1995. Nitrogen leaching, N uptake efficiency and water use from citrus trees fertilized at three N rates. Proc. Fl. State Hort. Soc. 108:151–155.
- Syvertsen, J.P. and M.L. Smith. 1996. Nitrogen uptake efficiency and leaching losses from lysimeter-grown *Citrus* trees fertilized at three nitrogen rates. J. Amer. Soc. Hort. Sci. 121:57–62.
- Syvertsen, J.P., M.L. Smith, and J.C. Allen. 1981. Growth rate and water relations of citrus leaf flushes. Ann. Bot. 47:97–105.
- Syvertsen, J.P., J. Lloyd, and P.E. Kriedemann. 1988. Salinity and drought stress effects on ion concentration, water relations and photosynthesis characteristics of orchard citrus. Austral. J. Agr. Res. 39:619–627.
- Tucker, D.P.H., A.K. Alva, L.K. Jackson, and T.A. Wheaton (eds.). 1995. Nutrition of Florida citrus trees. SP-169. Univ. of Florida, Gainesville.
- Waring, R.H. 1991. Responses of evergreen trees to multiple stresses, p. 371–390. In: H.A. Mooney, W.E. Winner, and E.J. Pell (eds.). Responses of plants to multiple stresses. Academic Press, New York.
- White, J.W., J.A. Castillo, and J. Ehleringer. 1990. Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. Austral. J. Plant Physiol. 17:189–198.
- Zhang, J., L. Fins, and J.D. Marshall. 1994. Stable carbon isotope discrimination, photosynthetic gas exchange and growth differences among western larch families. Tree Physiol. 14:531–539.
- Zhang, J. and J.D. Marshall. 1994. Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. Can. J. For. Res. 24:92–99.