How Nitrogen Supply Affects Growth and Nitrogen Uptake, Use Efficiency, and Loss from *Citrus* Seedlings

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Additional index words. ammonium nitrate, $^{15}\text{NO}_3$, $^{15}\text{NH}_4$, N partitioning, CO_2 assimilation, leaching loss, uptake-efficiency, *Citrus aurantium*, *C. reticulata*, *C. volkameriana*, *Poncirus trifoliata* $\times$ *C. paradisi*

Abstract. We examined how N supply affected plant growth and N uptake, allocation and leaching losses from a fine sandy soil with four *Citrus* rootstock species. Seedlings of ‘Cleopatra’ mandarin (*Citrus reticulata* Blanco) and ‘Swingle’ citrumelo (*C. paradisi* $\times$ *P. trifoliata*) were grown in a glasshouse in 2.3-liter pots of Candler fine sand and fertilized weekly with a complete nutrient solution containing 200 mg N/liter (20 mg N/week). A single application of $^{15}\text{NH}_4^{15}\text{NO}_3$ (17.8% atom excess ^{15}N) was substituted for a normal weekly N application when the seedlings were 22 weeks old (day 0). Six replicate plants of each species were harvested at 0.5, 1.5, 3.5, 7, 11, and 30 days after ^{15}N application. In a second experiment, NH_4NO_3 was supplied at 18, 53, and 105 mg N/week to 14-week-old ‘Volkamer’ lemon (*C. volkameriana* Ten. & Pasq.) and sour orange (*C. aurantium* L.) seedlings in a complete nutrient solution for 8 weeks. A single application of $^{15}\text{NH}_4^{15}\text{NO}_3$ (23.0% ^{15}N) was substituted at 22 weeks (day 0), as in the first experiment, and seedlings harvested 3, 7, and 31 days after ^{15}N application. Nitrogen uptake and partitioning were similar among species within each rate, but were strongly influenced by total N supply and the N demand by new growth. There was no ^{15}N retranslocation to new tissue at the highest (105 mg N/week) rate, but N supplies below this rate limited plant growth without short-term ^{15}N reallocation from other tissues. Leaf N concentration increased linearly with N supply up to the highest rate, while leaf chlorophyll concentration did not increase above that at 53 mg N/week. Photosynthetic CO_2 assimilation was not limited by N in this study; leaf N concentration exceeded 100 mmol·m⁻² in all treatments. Thus, differences in net productivity at the higher N rates appeared to be a function of increased leaf area, but not of leaf N concentration. Hence, N use efficiency decreased significantly over the range of N supply, whether expressed either on a gas-exchange or dry weight basis. Mean plant ^{15}N uptake efficiencies after 31 days decreased from 60% to 47% of the ^{15}N applied at the 18, 20, and 53 mg N/week rates to less than 33% at the 105 mg N/week rate. Leaching losses increased with N rate, with plant growth rates and the subsequent N requirements of these *Citrus* species interacting with residual soil N and potential leaching loss.

Minimum N concentrations in irrigation solutions, required to support high growth rates of containerized *Citrus* trees, are between 10 and 25 mg N/liter in various substrates under warm, high-irradiance greenhouse conditions (Chapman and Liebig, 1937; Haas, 1937; Lea-Cox, 1989; Maust and Williamson, 1994). However, since fertilizer solutions were applied to excess to ensure leaching and to avoid the accumulation of salts in all these studies, actual N requirements of *Citrus* species are not known. Bitcover and Wander (1950) calculated (by difference from N applied and N leached) that from 22% to 44% of the applied ammonium- or nitrate-N, respectively, was taken up within 10 minutes by budded *Citrus* trees in gravel. This implied a very high N uptake efficiency, but their results ignored any other potential N losses. Nitrogen uptake-efficiency over 4 weeks in lemon cuttings was 72% of $^{15}\text{NH}_4\text{NO}_3$ applied at 97 mg N/week but only 39% when applied at 485 mg N/week (Wallace, 1954). Maust and Williamson (1994) assumed that all ^{15}N supplied to ‘Hamlin’ orange [*C.*

sinensis (L.) Osb.] budded on Carrizo citrange [*C. sinensis* (L.) Osb. $\times$ *Poncirus trifoliata* (L.) Raf.] seedlings would be taken up completely within 7 days, based on their fertilization regime. They concluded that 17 mg N/day (119 mg N/week) was the critical N concentration in solution to maintain adequate continuous growth of young trees on the two rootstock. No study has directly measured uptake rates of *Citrus* rootstock with varying growth rates, while simultaneously considering the effects of the absolute quantity of N (mg N/week) supplied to the plant over time on residual soil N and leaching losses.

Nitrogen deficiency can limit growth (Lea-Cox, 1989; Maust and Williamson, 1994; Wallace, 1954) and increase partitioning of carbohydrates and nutrient ions to roots (Tolley-Henry and Raper, 1991). This can be reflected in increased root growth, N acquisition capacity and N uptake per gram of root (Rufty et al., 1990). Nitrogen deficiency-induced alterations in the distribution of assimilates between the shoot and root have a strong and rapid effect on decreasing the rate of leaf area development (Radin and Boyer, 1982), more so than plant dry weight (Ingestad and Lund, 1979). A constant relative growth rate, along with increasing N supply, ensures that leaf nutrient concentrations will remain constant overtime (Ågren, 1985). Any decline in leaf N concentration can indicate that nutrient supply is limiting the maximum potential growth rate of plants (McDonald et al., 1991).

Low leaf N can also limit net photosynthetic assimilation of CO_2 [Aco.] (DeJong, 1982; DeJong and Phillips, 1981; Hesketh et al. 1981; Mooney et al., 1978; Syvertsen, 1987). Photosynthates are produced in proportion to the amount of N used to construct leaf area, chlorophyll (Sinclair, 1992), and in particular the large

Received for publication 27 Mar. 1995. Accepted for publication 19 Sept. 1995. Florida Agricultural Experiment Station Journal series no. R-004467. Research supported by the U.S. Geological Survey (USGS), Dept. of the Interior, under USGS award number 1408-000 1 -G 1905. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the U.S. government. J. Lea-Cox thanks the Univ. of Florida Foundation Herlong graduate assistant supplement for financial support. We thank Martin L. Smith, Jr., for invaluable assistance. 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.

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proportion of leaf N that is contained in ribulose-1,5-bisphosphate carboxylase-oxygenase [rubisco] (Moreno and Garcia-Martinez, 1984). Above a certain leaf N concentration, N does not limit Aco_2 (Lugg and Sinclair, 1981; Evans, 1989) and the functional relationship between increasing leaf N concentration and Aco_2 becomes less clear. Less than 10% of leaf rubisco is active at leaf N concentrations above 200 $\text{mmol}\cdot\text{m}^{-2}$, based on Aco_2 rates from sun leaves of *Citrus paradisi* and *C. limon* leaves (Lloyd et al., 1992).

Any N applied in excess of the amount of N taken up by *Citrus* species increases soil N concentration and the potential of leaching of NO_3 from the soil-plant system. The objectives of this greenhouse study were to define the N requirement of various *Citrus* rootstock species with contrasting growth rates, and to determine how N supply affected N partitioning, N use efficiency and the N leaching loss potential from sandy soils. These species represent rootstock that are commonly used throughout the world for adaptation to varying edaphic and environmental conditions, ultimately to improve fruit quality.

Materials and Methods

Experiment 1

Plant treatments. Seedlings of two *Citrus* rootstock species of contrasting growth rates, slow-growing 'Cleopatra' mandarin (*Citrus reticulata* Blanco = CM) and fast-growing 'Swingle' citrumelo [*C. paradisi* Macf. $\times$ *Poncirus trifoliata* (L.) Raf. = SC], were grown from seed in a glasshouse covered with 50% shade-cloth from March to October, 1990. Thirty-six, 16-week-old seedlings of each species were selected for uniformity and individually transplanted into 2.3-liter plastic pots of pasteurized Candler fine sand (Typic Quartzipsamments), which had been steam pasteurized 2 days previously at 120C for 8 h. All plants received a weekly application of about 250 ml of a commercial [20N-(urea, NH_4 , and NO_3)-8.7P-16.7K] nutrient solution (fertilization) at a concentration of 200 mg N/liter (about 50 mg N/week) for the next 4 weeks. The average ($n = 6$) void volume of the soil in the pots was determined to be 350 ml (i.e., the volume of air space in the soil) at container capacity. Plants were watered to excess every other day, following standard citrus nursery practice, so that leaching of the pots occurred between fertigations. Beginning 2 weeks before the ^{15}N application, 100 ml of a 1N-0.2P-1K nutrient solution, using NH_4NO_3 as the sole N source, was applied weekly at 200 mg N/liter (20 mg N/week) to each plant, substituting for a normal irrigation. During this period, average daily photosynthetic photon flux (PPF) was about 650 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, minimum glasshouse air temperatures ranged between 13–18C and maxima 25–34C, relative humidity (RH) varied between 30% to 100% and vapor pressure deficit (VPD) from 0–3.7 kpa.

Application of ^{15}N . On day 0, when the plants were 22 weeks old, a single application of 17.81910 atom excess $^{15}\text{NH}_4^{15}\text{NO}_3$ at 200 mg N/liter was substituted for the N in the normal weekly fertilization in 100 ml water (=20 mg total N per plant). Two days before the application of ^{15}N , pots were thoroughly leached with distilled water, and then individually weighed at container capacity, when completely drained. The 100 ml volume of the ^{15}N application was enough solution to bring the soil to near container capacity (by weight) but insufficient to result in any leaching from the pot. Pots were weighed every 2 days thereafter and the weight of water lost through evapotranspiration was replaced with distilled water without leaching occurring. Seedlings were arranged in a completely randomized design. Six of the 36 seedlings of each species were randomly selected for harvest at 0.5, 1.5, 3.5, 7, 11, and 30 days after ^{15}N application (day 0). Three fertigations were

applied to the remaining seedlings at days 8, 16 and 22, using ^{15}N -Nat 200 mg N/liter.

Plant analysis. All seedlings were tagged on day 1 just below the shoot tip to determine the new growth (NG) portion at each harvest day following the application of ^{15}N . In addition, the terminal leaves of the 12 seedlings that were selected on day 1 to be harvested on day 30 were additionally tagged on days 15 and 29. Individual leaf lengths and widths were measured on these days so that relative growth rates (RGRs) could be calculated. A regression equation ($r^2 = 0.97$) predicted leaf areas on all measurement days by regressing the leaf length and width measurements (Syvertsen, 1985) on day 29 with the individual leaf areas measured on day 30.

On each of the 6 harvest days, the fibrous root (FR), taproot (TR), stem (S), fully expanded mature leaves (ML), and new shoot growth (NG = young shoot plus associated immature leaves initiated since ^{15}N application) fractions of each plant were weighed after drying at 60C for 48 h. At each harvest, the FR length of each plant was determined before drying, using a line intercept method (Tennant, 1975). Tissue fractions were milled (Cyclotec 1093, Tecator, Sweden) and total N concentrations of well-mixed tissue samples were analyzed by combustion and gas chromatography using a carbon/nitrogen analyzer (NA 1500; Carlo Erba, Fison Instruments, Paramus, N.J.). A mass spectrometer (VG602E; Vacuum Generators, Winsford, England) connected in series to the nitrogen analyzer determined the ^{14}N : ^{15}N ratio. Total tissue ^{15}N contents (mg per tissue) were calculated using the method of Cabrera and Kisel (1989). Samples of about 250 mg of ML tissue were ashed for 5 h at 500C (Gaines and Mitchell, 1979) and concentrations of cations were determined by inductively coupled plasma (ICP) analysis on a Perkin-Elmer (Norwalk, Conn.) Plasma 40, using a sequential scanning method.

Leachate and soil analysis. Just before each harvest, pots were leached with 400 ml of distilled water and the leachate collected. This resulted in a leachate volume of about 200–300 ml, from which a 20 ml sub-sample was immediately frozen until analyzed for N. After leaching, about 100 g of well-mixed soil from each pot was frozen for subsequent N analysis. Inorganic soil N was extracted from 50 g of soil with two consecutive 75-ml aliquots of 2 M KCl (Bremner, 1965), which were then combined into a single 150-ml sample. The concentrations of total $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in the leachate fractions and KCl extracts were analyzed using a rapid flow calorimetric autoanalyzer (Clesceri et al., 1989). Since solid samples are required for ^{14}N : ^{15}N ratio determination by the mass spectrometer, $^{15}\text{NH}_4\text{-N}$ and $^{15}\text{NO}_3\text{-N}$ fractions were recovered in separate 5-ml aliquots of boric acid by steam distillation of the soil and leachate solutions (Bremner and Keeny, 1966; Hauck and Bremner, 1976). The boric acid aliquots were then reduced to dryness before analysis using the mass spectrometer.

Experiment 2

Plant treatments. This experiment duplicated Expt. 1, except two different rootstock species, again of contrasting growth rate, were fertilized with three different rates of N. Fifty-four 14-week-old seedlings each of slow-growing sour orange (*C. aurantium* L. = SO) and fast-growing 'Volkamer' lemon (*C. volkameriana* Ten and Pasq. = VL) were grown in 2.3-liter pots of pasteurized Candler fine sand in the glasshouse, covered with 50% shade-cloth, from March to October, 1991. Eighteen plants of each species were fertilized with NH_4NO_3 at either 17.5, 52.5, and 105 mg N/week, supplied in 350 ml of distilled water (= 50, 150, or 300 mg N/liter) for the next 8 weeks in a completely randomized design. Phosphorus, K and S were supplied to all seedlings at 50, 300, and 45 mg-liter $^{-1}$ respectively, in all solutions. All irrigations between

fertilizations were applied to excess, as in Expt. 1, to ensure leaching and avoid any accumulation of salts. Average daily PPF was about $700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, minimum glasshouse air temperatures ranged between 14–20°C and maxima 24–32°C, RH from 40% to 100%, and VPD from 0–3.3 kPa.

Application of ^{15}N . The ^{15}N was applied to all treatments exactly as described in 'Expt. 1. All pots were thoroughly leached and weighed at container capacity before the ^{15}N application, which again substituted for the ^{14}N in a normal weekly fertiligation when the plants were 22 weeks old. The isotopic enrichment of the $^{15}\text{NH}_4^{15}\text{NO}_3$ varied slightly for each rate, i.e., 23.43%, 23.03%, and 22.8% atom excess for the 18, 53, and 105 mg treatments, respectively. Additional complete fertilizations at each rate were applied (using ^{14}N) on days 7 (after plant harvest), 15, and 23 without leaching. Plant dry weight analysis and nutrient ICP analyses after harvest were determined as in Expt. 1.

Plant analysis. Six randomly selected seedlings of each species and N rate were harvested on days 3, 7, and 31 after ^{15}N application (day 0). The terminal leaves of all seedlings were tagged on day 1 as in Expt. 1. In addition, the leaves of the 18 seedlings of each species randomly assigned at day 0 to the 31-day harvest were retagged on days 11, 18, and 30. Individual leaf lengths and widths

were measured on these days so that relative growth rates could be calculated using predicted leaf areas, as described in Expt. 1.

The youngest fully expanded leaf, selected on day 1, of each seedling to be harvested on day 31 was tagged for repeated measurement of Aco_2 on days 17, 18, 25, and 28, performed with a portable photosynthesis system (6200; LI-COR, Lincoln, Neb.) with a 1-liter measurement cuvette operated in closed mode. All measurements were made between 0900–1200 HR when photon flux density (PPF) levels were between $700\text{--}1400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (above light saturation). Leaf temperatures in the cuvette ranged from 31–37°C with leaf-to-air vapor pressure differences from 2.2–3.6 kPa. Total leaf chlorophyll concentrations were determined (Moran and Porath, 1980) from two 10-mm discs removed at harvest on day 31 from mid-lamina of the leaf used for gas-exchange measurements.

Whole-plant transpiration per unit leaf area was calculated from daily weight loss of the 31-day-harvest seedlings on days 17, 18, 25, 28, and 30. Root hydraulic conductivity of each species at the 18 and 53 mg N rates were measured on day 31, using a pressure chamber technique (Graham and Syvertsen, 1984). Briefly, the plant stem was cut 6 cm above the soil line and the pot with intact root system pressurized to a constant 0.38 MPa. After an initial equilibration time of 15 min, the weight of xylem fluid that exuded from the cut stump during three 5-min periods was measured. The hydraulic conductivity of roots was expressed as the weight of exudate per unit length of fibrous root per unit time and pressure ($\mu\text{g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$).

Leachate and soil analysis. Leachate and soil analysis were as in Expt. 1, except that pots were leached with a larger volume (930 ml) of distilled water to ensure total leaching. The electrical conductivity (EC) of all leachates was measured. Soil temperatures were recorded at 0.5-h intervals throughout the 30-day period using an Omnidata (Logan, Utah) datapod (model DP211) with a thermistor probe in the middle of the pot. Overall, soil temperatures lagged behind air temperatures by about 3 h, between the range 14 to 30°C.

Statistical analysis. Plant tissue dry weight and N data from Expts. 1 and 2 were each analyzed separately. Within each experiment, data were tested for significant differences within each harvest date, using a factorial analysis of variance with six replicate seedlings per treatment in a completely randomized design. When a significant ($P < 0.05$) interaction between rates occurred in the ANOVA, regression analysis was used to determine the relationship between individual treatments (Montgomery, 1984). Percentage data from each experiment were transformed before analysis by taking the arcsine of the square root of the value (Ott, 1988). Gas exchange and WPT data (Expt. 2) were analyzed using a repeated measures analysis (Littell, 1989), since all data were collected from the same plants on repeated days.

Results

Growth. For ease of comparison, the rootstock species were grouped in some tables and figures according to their respective growth rates. Where appropriate, t tests were used to compare results between experiments. 'Cleopatra' mandarin and SO had smaller total plant dry weight increases over the ^{15}N uptake period (Fig. 1a) than SC and VL seedlings of the same age (Fig. 1b) at all N rates, even though the initial dry weights of all seedlings were comparable at day 3. There was no difference in total dry weight among species of similar growth rate over the experimental period at 20 mg or 18 mg N/week (parallel slopes in Fig. 1a and b), but the higher N rates produced greater dry weights (Fig. 1a and b) and

Fig. 1. Total plant dry weight ($n=6$) of (a) 'Cleopatra' mandarin (CM) and sour orange (SO), (b) 'Swingle' citrumelo (SC) and 'Volkamer' lemon (VL) seedlings; new leaf area ($n=6$) of (c) CM, SO seedlings, and (d) SC, VL seedlings for 31 days after application of ^{15}N at each respective N rate (mg N/week). "Nitrogen applications at each rate indicated on the x-axis by small arrows (Expt. 1) and large arrows (Expt. 2). Error bars represent 1 SE from the mean. No error bar indicates a SE smaller than the symbol size.

Table 1. Shoot: root ratio (dry weight basis), new leaf growth dry weight: leaf area ratio (DW/LA), fibrous root (FR) length, and specific root length (SRL) of Cleopatra mandarin (CM), Swingle citrumelo (SC) seedlings (n = 6) in Expt. 1, and sour orange (SO), Volkamer lemon (VL) seedlings in Expt. 2 at day 31.

Species-N rate	Shoot : root (g·g ⁻¹)	New leaf growth		FR length (m)	FR SRL (m·g ⁻¹)
		DW/LA (g·m ⁻²)			
CM-20 mg	3.18	83.8		17.03	2.06
SC-20 mg	2.03	72.0		21.95	1.30
<i>t</i> test: (<i>P</i> values)					
Species	<0.001	<0.001		<0.001	<0.001
SO-18 mg	1.87	62.7		24.08	1.67
SO-53 mg	3.01	59.6		27.87	2.05
SO-105 mg	2.60	66.6		25.13	1.69
VL-18 mg	2.05	54.1		34.99	1.99
VL-53 mg	2.95	57.4		39.36	2.19
VL-105 mg	2.62	63.9		35.64	1.77
Anova: (<i>P</i> values)					
Species	0.65	0.02		<0.001	<0.001
Rate	<0.001	<0.01		0.42	<0.001
Species × rate	0.62	0.25		0.99	0.10

more leaf area (Fig. 1 c and d). Although VL and SC were larger than CM and SO by day 31, total plant dry weight of plants did not differ within each group, whether fertilized at the 53 or 105 mg N/week rates (Fig. 1 a and b).

SO and VL in Expt. 2 developed more new leaf area (Figs. 1 c and d) and increased shoot : root ratio (Table 1) with an increase in N rate. The reduction in new leaf area of both species at the 105 mg rate by day 31 (Table 1) was associated with a significant ($P < 0.01$) increase in dry weight: leaf area (DW/LA) ratio at this N rate (Table 1). The relative growth rates of SO and VL leaves between days 18 and 30 were lower ($P < 0.05$) for the 105 mg rate (0.11 and 0.18 cm²·cm⁻² per day) than at the 18 or 53 mg rates (0.25 and 0.20, 0.21, and 0.27 cm²·cm⁻² per day, respectively). The leaf-to-plant dry weight ratio decreased at total plant N contents above 220 mg in SO and above about 300 mg N for VL (Fig. 2a), indicating declining leaf growth rate in both rootstock species, despite N being available.

The smaller shoot: root ratio of SC in Expt. 1 reflected the lower specific fibrous root length (SRL) of this species compared to CM, but there was no significant effect of N rate on FR length between SO and VL in Expt. 2 (Table 1). There was, however, a significant ($P < 0.001$) reduction in FR/total plant dry weight ratio for slow- and fast-growing rootstock species, with increasing plant N content (Fig. 2b). Fibrous root SRL was significantly higher (by *t* test) for SO and VL at 53 mg N/week than at either 18 or 105 mg N/week by day 31 (Table 1).

Water relations. Whole plant transpiration (WPT), which integrated plant water use from 0800 to 1800 HR on various measurement days throughout the 31-day period in Expt. 2, showed an expected variation between days, due primarily to varying temperatures and vapor pressure deficits between measurement days (data not shown). Typical data (day 28) are shown, indicating that, on average, VL had higher total transpiration than SO, particularly at the 53 and 105 mg rates (Table 2). The WPT was significantly ($P = 0.02, 0.06$) higher at the 18 mg N/week rate for SO and VL, than at the 53 or 105 mg N/week rates. Root hydraulic conductivities measured at day 31 were significantly lower ($P < 0.01$, by *t* test) at the 18 mg rate in comparison to 53 mg N/week for VL but not SO (Table 2).

Elemental analysis. Total N concentrations of mature leaves

from plants in Expt. 1 always exceeded 30 mg·g⁻¹ for CM and SC during the 3 1-day uptake period in Expt. 1. In Expt. 2, leaf N

Fig. 2. (a) Leaf and (b) fibrous root to plant dry weight ratios (n = 6) of sour orange (SO) and 'Volkamer' lemon (VL) seedlings (Expt. 2), 31 days after application of N at each respective N rate (mg N/week).

Table 2. Whole-plant transpiration (WPT) on day 28, root conductivity, mature leaf (ML) nitrogen, and total (ah) chlorophyll concentrations for sour orange (SO) and 'Volkamer' lemon (VL) seedlings (n = 6) in Expt. 2.

Species-N rate	WPT (day 28) (mg·m ⁻² ·s ⁻¹)	Root conductivity (μg·m ⁻² ·s ⁻¹ ·MPa ⁻¹)	ML N (μmol·m ⁻²)	ML chlorophyll (μmol·m ⁻²)
SO-18 mg	32.5	6.0	126	285
SO-53 mg	23.2	8.5	170	401
SO-105 mg	23.9	N M	250	351
Linear	***		***	NS
Quadratic	**		***	NS
VL-18 mg	32.3	14.1	136	308
VL-53 mg	28.5	29.3	184	425
VL-105 mg	28.0	NM	214	434
Linear	*		***	***
Quadratic	*		***	**
Anova: (P values)				
Species	<0.001	<0.001	0.66	<0.001
Rate	<0.01	<0.01	<0.001	<0.001
Species × rate	0.06	0.04	0.10	0.05

NM = not measured; mean separation by t test.

NS,*,*** Nonignificant or significant at $P < 0.10$, 0.05 , or 0.01 , respectively.

concentrations ranged from 24–28 mg·g⁻¹ DW at the lowest N rate, to 29–38 and 36–43 mg·g⁻¹ at 53 and 105 mg N/week, respectively

Fig. 3. Whole-plant N uptake for (a) 'Cleopatra' mandarin (CM) and sour orange (SO), (b) 'Swingle' citrumelo (SC) and 'Volkamer' lemon (VL) seedlings (n = 6) during the 7 days after application of ¹⁵N at each respective N rate (mg N/week). Nitrogen applications at each rate indicated on the x-axis by small arrow (Expt. 1) and large arrow (Expt. 2). Error bars represent 1 SE from the mean.

(data not shown). Leaf nutrient concentrations of *Citrus* species having different growth rates, however, can be misleading (Lea-Cox and Syvertsen, 1993). Total leaf N content was, therefore, calculated by multiplying N concentration by leaf dry weight. Since SO and VL had similar mature leaf dry weights at each N rate on day 3 (Lea-Cox, 1993), N content followed the same pattern on day 3 as N concentration. By day 31, however, VL fertilized at the highest rate had significantly ($P < 0.01$) more leaf N (118 mg) than SO (82 mg) due in large part to the greater leaf dry weight of VL (2.8 g) than SO (2.3 g). This increase in N content at day 31 was reflected in the mature leaf N concentration of VL (43 mg·g⁻¹), but not for SO (35 mg·g⁻¹).

The macroelement concentrations of CM and SC in Expt. 1 were all in the sufficient range (data not shown) for mature *Citrus* trees (Koo et al., 1984; Smith, 1966b), although there is not enough data in the literature to indicate whether these ranges are applicable to young seedlings of different growth rates. The concentrations of leaf P and K in Expt. 2 were in the optimum to high range (1.7–1.9 mg·g⁻¹ P; 16–19 mg·g⁻¹ K) for SO and VL at all harvests. 'Volkamer' lemon leaf Ca (17–20 mg·g⁻¹) and Mg (2.3–2.5 mg·g⁻¹) were in the low range, but were more concentrated for SO (20–22 and 3.0–3.7 mg·g⁻¹, respectively), due to the smaller total leaf area of this species (Table 1).

Nitrogen uptake. Total N uptake rates on days 3 and 7 (Fig. 3) were calculated by multiplying ¹⁵N uptake (Figs. 4 and 5) by the reciprocal of the atom excess ¹⁵N applied (e.g., 4.906 mg total ¹⁵N × 1/0.2303 for SO-53 mg at day 7). This calculation assumes that there was minimal residual ¹⁵N in the soil pool, since all treatment soils were thoroughly leached with distilled water before ¹⁵N application at day 0. Nitrogen uptake at 18 and 20 mg N/week was significantly lower than at the higher rates (Fig. 3), and appeared to be N limited, because little further ¹⁵N was taken up after 3 days by SO (Fig. 3a) or after 7 days by VL (Fig. 3b). All four rootstock took up similar amounts on N (50% to 60% = 9 to 12 mg ¹⁵N) at the 18 and 20 mg rates (Figs. 4 c and d and 5 c and d) by 31 days. Total N uptake at 53 and 105 mg N/week was similar for SO and VL over the first 7 days. However, uptake of ¹⁵N continued over the entire 31-day period at the 105 mg N rate for SO (Fig. 4a) and especially by VL (Fig. 5a), despite further applications of ¹⁵N, which would have diluted the residual ¹⁵N in the soil. By day 31, the total amount

Fig. 4. ¹⁵Nitrogen uptake into new growth, mature leaf, stem, taproot and fibrous root tissues (n = 6) at 3, 7 and 31 days after ¹⁵N application for sour orange (SO) seedlings (Expt. 2) and at 0.5, 1.5, 3.5, 7, 11, and 30 days for 'Cleopatra' mandarin (CM) seedlings (Expt. 1), at each respective N rate (mg N/week). Percentage of applied ¹⁵N taken up at each rate is indicated for each tissue. ¹⁵Nitrogen applications at each rate indicated on the x-axis by large arrows. Error bars represent 1 SE from the mean.

of N (i.e., total N concentration x total dry weight) in SO and VL at the 105 mg treatment was greater than the 53 mg treatment, i.e., 28.3 vs. 24.8 mg and 34.2 vs. 26.2 mg, respectively. Despite this, the overall uptake efficiency of SO (sum of percentage allocation, Fig. 4) was lowest for the 105 mg treatment (26.8%, Fig. 4a) compared to that of the 53 and 18 mg treatments (46.8% and 50.9%, Fig. 4 b and c, respectively). However, VL at 105 mg N/week took up significantly more N (i.e., Σ % allocation = $0.326 \times 105 = 34.2$ mg) by day 31 than all other treatments (Fig. 5a), reflecting the demand of its greater dry mass accumulation over the period (Fig. 1b).

Nitrogen partitioning. Species differences in ¹⁵N partitioning were evident at the lowest N rates in both experiments (Figs. 4 c and d and 5 c and d), but differences diminished at the higher rates in Expt. 2 (Figs. 4 a and b and 5 a and b). A greater proportion of ¹⁵N was partitioned into new growth of both species at the 18 and 53 mg rates than at the 105 mg rate, as evidenced by a large reduction of ¹⁵N content in all other tissues at the 18 and 53 mg treatments for both species by 31 days. Such a reallocation did not occur in the 105 mg treatment, where all tissues maintained or increased their ¹⁵N contents over the time period (Figs. 4a and 5a). Similar amounts [17.6 to 18.3 mg (i.e., $21.9\% + 12.7\% \times 53 \text{ mg} = 18.3$)] of ¹⁵N were allocated into leaf tissue (new growth and mature leaves) of the 53 and 105 mg rate treatments for SO and VL.

Fig. 5. ¹⁵Nitrogen uptake into new growth, mature leaf, stem, taproot and fibrous root tissues (n = 6) at 3, 7, and 31 days after ¹⁵N application for 'Volkamer' lemon (VL) seedlings (Expt. 2) and at 0.5, 1.5, 3.5, 7, 11 and 30 days for 'Swingle' citrumelo (SC) seedlings (Expt. 1), at each respective N rate (mg N/week). Percentage of applied ¹⁵N taken up at each rate on the final day is indicated for each tissue. ¹⁵Nitrogen applications at each rate indicated on the x-axis by large arrows. Error bars represent 1 SE from the mean.

Leaf N and chlorophyll. Mature leaf N concentrations were significantly higher at the 105 mg rate than for either the 18 or 53 mg rates for both species (Table 2). Total mature leaf chlorophyll concentration on a fresh weight basis ($\mu\text{mol}\cdot\text{m}^{-2}$), however, exhibited no significant increase at rates above 53 mg N/week. Sour orange chlorophyll concentrations decreased at the highest N supply rate, such that the regression analysis showed no significant linear or quadratic effects (Table 2).

Net photosynthetic CO₂ assimilation. Net photosynthetic CO₂ assimilation showed no significant variation between days, and so only the results from a typical day (day 28) are presented. Leaf N concentration did not apparently limit ACO₂ since there was no difference in ACO₂ between N rate or species (Fig. 6a). The phyllotaxy of 6-month-old seedlings and the spacing of the seedlings in the greenhouse minimized shading effects. Whole-plant ACO₂ rates were calculated by multiplying individual leaf ACO₂ (day 28) rates by their respective total plant leaf areas on day 31, solely to assess whether differences in treatment leaf areas could have a large effect on the total assimilation of C by the plant. Figure 6b indicates that total ACO₂ per plant increased at a greater rate for SO compared to VL with increases in total leaf N up to the 53 mg N/week rate.

Nitrogen-use efficiency. The increase in mature leaf DW/LA ratio between treatments was taken into account by the calculation

Fig. 6. (a) Mature leaf CO₂ assimilation rates on a representative measurement day (day 28) for sour orange (SO) and 'Volkamer' lemon (VL) seedlings (n = 6) at each respective N rate (mg N/week) versus mature leaf N concentration (leaf area basis); (b) Net plant assimilation (leaf ACO₂ × total leaf area) for sour orange (SO) and 'Volkamer' lemon (VL) seedlings (n = 6) on a representative measurement day (day 28) for each respective N rate (mg N/week) versus total leaf N content.

of N use efficiency on a gas exchange basis (NUE_A; Mooney and Gulmon, 1979). The NUE_A decreased as mature leaf N concentrations increased (Fig. 7a) above 100 mmol·m⁻² (about 23 mg·g⁻¹ DW). There were few species differences in this relationship, except for the greater range in N concentration for VL (Fig. 7a). When N use efficiencies of SO and VL were calculated on a total dry weight basis (NUE_W) and plotted against total plant N content (Fig. 7b), mean NUE_W decreased at the same rate as when leaf NUE_A was plotted against leaf N concentration (Fig. 7a). Although the relatively slower-growing SO had lower total dry weight and hence total plant N, NUE_W decreased at virtually the same rate as VL (Fig. 7b).

Nitrogen loss. Plant ¹⁵N uptake accounted for nearly 60% of the applied ¹⁵N after 30 days for both rootstock species in Expt. 1 (Table 3). Almost all the ¹⁵N recovered from the soil or leached water after day 1.5 was as ¹⁵NO₃, and soil ¹⁵NO₃ recoveries were minimal after the first 7 days. Total recoveries of the ¹⁵N applied accounted for about 50% at day 3.5 and 70% of the ¹⁵N at day 31. Poor total recoveries early in Expt. 1 were probably due to the small volume (400 ml) of water applied to leach the soil. Soil ¹⁵NH₄ recoveries in Expt. 2 (Tables 4 and 5) were substantially greater at day 3 in comparison to those recovered in Expt. 1 at day 3, particularly for the slower-growing SO species and at the higher rates of fertilization.

Fig. 7. (a) Gas exchange nitrogen use efficiency (NUE_A) per mmol leaf N for sour orange (SO) and Volkamer lemon (VL) seedlings (n = 6) for each respective N rate (mg N/week) versus leaf N concentration; (b) Dry weight nitrogen-use efficiency (NUE_W) per gram total plant dry weight for 'Cleopatra' mandarin (CM), sour orange (SO), 'Swingle' citrumelo (SC) and 'Volkamer' lemon (VL) seedlings (n = 6) for each respective N rate (mg N/week) versus total plant N content.

In Expt. 2, significantly less soil ¹⁵NH₄ was recovered from VL at the two lower N application rates (Table 5) compared to SO (Table 4). Soil ¹⁵NO₃ recovery was high at day 3 but declined thereafter in the 18 and 53 mg treatments. Less of a decrease was observed at the 105 mg N/week rate. Nitrate-¹⁵N increased significantly in the leached water of the 105 mg rate (counter to the trend in the other two treatments) after day 3, perhaps indicating significant soil vitrification (Lea-Cox, 1993), since the KCl-extractable soil ¹⁵NH₄ decreased significantly at 105 mg N/week during this time. Between 10% and 40% of the ¹⁵NO₃, either directly from the applied ¹⁵NO₃, or nitrified from ¹⁵NH₄ was leached from the pot within the first 3 to 3.5 days from all treatments in both experiments (Tables 3, 4, and 5). The effect of increasing N rate in Expt. 2 was most noticeable at day 31 when negligible amounts of ¹⁵N were leached from the 18 mg treatment (Tables 4 and 5), whereas increasing amounts (14% to 21% and 46% to 60% for the 53 mg and 105 mg treatments, respectively) of the total applied ¹⁵N were leached as ¹⁵NO₃ from the soil. Electrical conductivities were 0.36 (±0.07), 0.75 (±0.05), and 1.88 (±0.19) dS·m⁻¹ for leachate volumes (793 ± 56 ml) from the 18, 53, and 105 mg N/week treatments respectively, at day 31. Total N recoveries exceeded 100% of that applied at the highest N rate on day 3, reflecting the large variability

Table 3. Percentage of applied ^{15}N recovered from leachate, soil, and plant fractions of 'Cleopatra' mandarin (CM) and 'Swingle' citrumelo (SC) seedlings ($n = 6$) 0.5 to 31 days after application of ^{15}N , at 20 mg total N/week in Expt. 1. Soil and leached NH_4 and NO_3 budgets presented as percentage recovered from N applied as $0.5^{15}\text{N H}_4\text{-N} : 0.5^{15}\text{N O}_3\text{-N}$ at Day 0. Numbers in parentheses after the totals represent 1 SE about the mean.

Species-N rate Days	Percentage						Total
	Plant	Soil NH_4	Soil NO_3	Leached NH_4	Leached NO_3		
CM-20 mg	0.5	2.7	1.6	3.4	0.1	13.4	21.2 (± 3.5)
	1.5	4.1	1.4	3.4	0.1	11.4	20.3 (± 1.9)
	3.5	12.1	0.0	1.0	0.0	38.9	52.0 (± 3.5)
	7	32.3	0.1	0.4	0.1	34.3	67.1 (± 3.4)
	11	33.4	0.1	0.6	0.2	27.7	61.9 (± 4.5)
	31	60.0	0.0	0.1	0.0	7.3	67.4 (± 2.6)
SC-20 mg	0.5	6.3	0.6	5.1	0.1	12.2	24.2 (± 3.2)
	1.5	7.9	0.8	3.0	1.4	14.8	27.9 (± 2.3)
	3.5	19.2	0.0	1.5	0.2	28.5	49.5 (± 1.6)
	7	38.4	0.1	0.2	0.2	34.7	73.7 (± 5.1)
	11	39.4	0.1	0.5	0.7	25.2	65.8 (± 4.8)
	31	58.7	0.0	0.0	0.3	13.0	72.0 (± 2.8)
Anova: (<i>P</i> values)							
Species		<0.001	0.92	0.59	0.27	0.98	
Time		<0.01	0.33	<0.001	0.96	<0.001	
Species $\times$ time		0.94	0.99	0.14	0.97	0.14	

($\pm 20\%$ to 30%) of the soil NH_4 data for SO and VL (Tables 4 and 5).

Green algae grew on the soil surface during the first 8 weeks in Expt. 2, particularly on those pots fertilized at the highest rate. This organic material was scraped off the soil surface before application of ^{15}N but may have been indicative of an increase in microorganism activity in the soil (Lea-Cox, 1993). In a related observation, water leached from soils fertilized at the 105 mg rate which had the highest EC, consistently appeared clear in comparison to the increased turbidity of the leachates from the 53 mg and especially the 18 mg N/week treatment.

Discussion

Growth of 6-month-old SO and VL seedlings was limited by N supply at 18 mg N/week, regardless of their different growth rates. Leaf N concentrations of $25 \text{ mg} \cdot \text{g}^{-1}$ were maintained at this low N

rate in both rootstock species because of the limited plant growth and lower leaf area than the higher N treatments. Total plant dry weight of SO and VL increased at 53 and 105 mg N/week, with no difference between these rates, even though mature leaf N concentrations were higher at the 105 mg N/week rate. New leaf growth at N concentrations above 18 mg N/week appeared to be a driving variable for N uptake. There was no significant increase in total leaf area or total plant dry weight above 53 mg N/week, although leaf N concentration and total plant N was greater at the 105 mg rate by the end of the study. Thus, there appeared to be a considerable increase in N uptake at the 105 mg rate, without new vegetative growth. There was, however, an increase in leaf weight per unit area at this rate and hence the N concentration of these leaves increased on an area basis with increasing N availability.

The larger shoot : root ratios of slower- and faster-growing species at higher N rates is consistent with data in the literature

Table 4. Percentage of applied ^{15}N recovered from leachate, soil, and plant fractions of sour orange (SO) seedlings ($n = 6$) over 31 days, at three N rates (18, 53 and 105 mg total N/week) in Expt. 2. Soil and leached NH_4 and NO_3 budgets presented as percentage recovered from N applied as $0.5^{15}\text{N H}_4\text{-N} : 0.5^{15}\text{N O}_3\text{-N}$ at day 0. Numbers in parentheses after the totals represent 1 SE about the mean.

Species-N rate Days	Percentage						Total
	Plant	Soil NH_4	Soil NO_3	Leached NH_4	Leached NO_3		
SO-18 mg	3	44.2	13.0	11.2	2.4	28.2	99.0 (± 13.5)
	7	44.0	12.8	0	0.2	5.0	62.0 (± 4.9)
	31	50.9	5.5	1.2	0	1.0	58.6 (± 2.5)
SO-53 mg	3	20.4	22.8	17.9	2.9	34.5	98.5 (± 7.5)
	7	40.6	3.1	2.5	0.2	28.7	75.1 (± 4.0)
	31	46.8	0	1.2	0.3	21.3	69.6 (± 2.5)
SO-105 mg	3	11.5	57.4	17.7	7.2	28.4	122.2 (± 9.8)
	7	19.0	6.5	8.9	9.9	47.7	92.0 (± 7.0)
	31	26.8	6.2	1.8	7.8	45.5	88.2 (± 6.6)
Anova: (<i>P</i> values)							
Time		<0.001	<0.001	<0.001	<0.01	<0.01	
Rate		<0.001	<0.001	<0.01	<0.001	<0.001	
Time $\times$ rate		0.50	<0.01	0.31	0.02	<0.001	

Table 5. Percentage of applied ^{15}N recovered from leachate, soil, and plant fractions of 'Volkamer' lemon (VL) seedlings ($n = 6$) over 31 days, at three N rates (18, 53, and 105 mg total N/week) in Expt. 2. Soil and leached NH_4 and NO_3 budgets presented as percentage recovered from N applied as 0.5 $^{15}\text{NH}_4\text{-N}$:0.5 $^{15}\text{NO}_3\text{-N}$ at day 0. Numbers in parentheses after the totals represent 1 SE about the mean.

Species–N rate Days	Percentage					
	Plant	Soil NH_4	Soil NO_3	Leached	NH_4 Leached	NO_3 Total
VL–18 mg	3	40.7	0	11.6	0.2	21.5 74.0 (± 7.0)
	7	54.5	3.6	0	0.2	4.1 62.4 (± 3.7)
	31	49.9	1.1	0	0	0.8 51.8 (± 1.6)
VL–53 mg	3	26.7	4.6	34.4	2.9	28.9 97.5 (± 11.4)
	7	40.9	0.5	0.5	0.1	21.6 63.6 (± 4.6)
	31	49.4	0.7	1.0	0.3	14.4 65.8 (± 4.6)
VL–105 mg	3	11.2	45.2	19.6	11.6	40.1 127.7 (± 11.1)
	7	18.1	1.9	6.5	10.2	55.3 92.8 (± 4.4)
	31	32.6	2.9	9.8	3.7	59.8 108.8 (± 4.4)
Anova: (P values)						
Time	<0.001	<0.001	<0.001	<0.001	0.02	
Rate	<0.001	<0.001	<0.001	<0.001	<0.001	
Time $\times$ rate	0.44	<0.001	<0.01	<0.001	<0.001	

where the proportion of dry weight allocated to roots decreases under high N availability (Tolley–Henry and Raper, 1991). The decrease in relative growth rate, shoot : root ratio, specific root length and total leaf area of SO and VL at the 105 mg N rate may have been a function of the periodicity of shoot growth or resource allocation in this treatment over the 4-week period. Leaf DW/LA ratio increased with N rate, increasing N storage in the leaf of both species by day 31, which may have also reflected the leaf aging process (Syvertsen et al., 1981).

The similarity in the uptake and partitioning of N among the various species within each rate was remarkable considering their differences in growth rate. Despite higher leaf N concentrations for CM and SC fertilized initially at 50 and then 20 mg N/week (Expt. 1) in comparison to SO and VL at 18 mg N/week (Expt. 2), total ^{15}N uptake was similar for all four species by day 31. More importantly, leaf N concentrations of plants increased with N rate, and were maintained throughout the 31 days. Thus, N supply above 53 mg N/week was sufficient to maintain a high average RGR for SO and VL seedlings. At the 105 mg N/week rate, the ^{15}N uptake was adequate to maintain the ^{15}N content of all tissues and support the new growth requirements with the extra N going into storage reflected in increased leaf thickness. At 53 mg N/week, however, reallocation of ^{15}N from ML and other tissues was necessary to support the demand of the new vegetative growth. This may indicate that 53 mg N/week over a longer-term or under different environmental conditions may have been insufficient to support as much growth as 105 mg N/week.

It is possible that the extra N content of leaves at the 105 mg rate provided an additional benefit in increasing chlorophyll content (at least in VL) and hence the long-term NUE of the plants. Nitrogen in leaves constitutes a large part of the N reserves of *Citrus* trees (Moreno and Garcia-Martinez, 1984). Evans (1989) indicated that leaf chlorophyll concentration increases linearly with total leaf N from 50–200 mmol·m⁻² for a number of different species grown under high light conditions with variable N nutrition. Our data indicate that this relationship is asymptotic, as the increase in leaf N concentration over this range did not increase leaf chlorophyll concentration in SO. When total leaf areas per plant were used to estimate whole-plant assimilation, it was clear that high N supply increased total plant assimilation by increasing the leaf area. The significant decrease in N use efficiencies indicate that at least

under these conditions, the ACO_2 of *Citrus* species did not increase with increasing leaf N above a threshold of 100 mmol·m⁻² (about 23 mg·g⁻¹) (Syvertsen, 1987). These data support the contention of Lloyd et al. (1992) that low NUE_a in *Citrus* species may be a consequence of decreasing proportions of leaf N being allocated to the photosynthetic process as N concentration increases.

Available N (from the single ^{15}N application) continued to be taken up over a 31-day period, even though additional ^{15}N applications diluted the tracer. The significant increases in leaf N concentrations of SO and VL were a result of the additional N fertigations, since a lack of leaching between fertigations increased soil N availability. Nevertheless, all N uptake rates reported here are considerably lower than those suggested by Bitcover and Wander (1950) and show that N uptake is strongly dependent on the relative growth rate of the species and total N availability. The trees in their study were much larger, however, and total nutrient uptake is obviously correlated to tree size. The efficiency of uptake was inversely related to the amount supplied, which has been generally observed in field experiments (Smith, 1966a). Extrapolation of the uptake-efficiencies to 59 days [e.g. (about 30% $\times$ (105 mg) $\times$ 4 applications $\times$ 59/31 days) = 240 mg] shows that our data were very similar to those of Wallace (1954) [i.e. (39% $\times$ 485 mg) = 190 mg].

Half of the ^{15}N applied was in the form of $^{15}\text{NH}_4^+$. The percentage of residual soil $^{15}\text{NH}_4^+$ decreased to very low levels within 3 days in Expt. 1 and 7 days (depending on rate) in Expt. 2. This suggested either rapid preferential plant uptake of NH_4^+ , microbial conversion to NO_3^- , and/or adsorption to soil organic matter (Ardakani et al., 1973). A greater proportion of the ^{15}N was found as $^{15}\text{NO}_3^-$ than as $^{15}\text{NH}_4^+$ in the leached water, particularly at the highest (105 mg N/week) rate. A large proportion of the ^{15}N applied, 12910 to 48%, could not be accounted for in these experiments, particularly after the first 7 days and at the lower N application rates. Similar fractions have been unaccounted for in other ^{15}N experiments (Broadbent and Clark, 1965) and with mature *Citrus* ^{15}N experiments (Dasberg, 1987, Lea–Cox and Syvertsen, 1992). Insufficient leaching of the soil profiles probably contributed to the poor recoveries of ^{15}N from the first two harvests in Expt. 1. The small leaching volume used in Expt. 1 (400 ml) may have been insufficient to elute the entire soil volume, even though this volume was 115% of the average void volume of the pot. As the ^{15}N moved lower through the soil profile before later

harvests, it was more likely to be eluted by this leaching application of distilled water. In addition, the surface growth of algae implies that there may have been increased microbial competition for N in the soils at the lower N rates (Lea-Cox, 1993).

Coupled with the plant growth data, the large amount of NO₃ in the leached water at the higher rates provided evidence that N availability at the 53 and 105 mg rates did not limit plant growth or the rate of N uptake. The potential for leaching losses from N applications above those immediately required for plant growth therefore, appears high in small volume containers of sand. Increasing the N supply above 53 mg N/week apparently only increased the residual soil N and the likelihood of N being leached below the root zone, for slow (SO) and fast-growing (VL) rootstock. Maust and Williamson (1994) stated that the critical concentration for the growth of young budded *Citrus* rootstock was between 15 and 19 mg N/liter. They applied about 1 liter of their nutrient solutions on a daily basis, which equates to about 105–133 mg N/week. In reconciling these two studies, it is apparent that about 105 mg N/week maybe required for maintaining the growth of faster-growing rootstock, but excessive for slower-growing species. In addition, a substantial portion of the N at this rate is able to be leached, if applied on a weekly basis. Supplying low concentrations of N daily (i.e., 8–15 mg N/day per seedling) should, however, substantially reduce N leaching from sandy soils, and maintain the high growth rates of *Citrus* seedlings up to 8 months old.

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