Nitrogen Uptake by *Citrus* Leaves

John D. Lea-Cox¹ and James P. Syvertsen

Citrus Research and Education Center, University of Florida-IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850-2299

Additional index words. K¹⁵NO₃, ¹⁵N-urea, foliar-uptake, translocation, phytotoxicity, *Citrus paradisi* 'Redblush'

Abstract. We studied whether foliar-applied N uptake from a single application of low-biuret ¹⁵N-urea or K¹⁵NO₃ to citrus leaves was affected by N source, leaf age, or whole-shoot N content. In a glasshouse experiment using potted 18-month-old *Citrus paradisi* (L.) 'Redblush' grapefruit trees grown in full sun, 2- and 6-month-old leaves on single shoots were dipped into a 11.2 g N/liter (1.776% atom excess ¹⁵N-urea) solution with 0.1% (v/v) Triton X-77. Two entire trees were harvested 1.5, 6, 24, and 48 hours after ¹⁵N application. Uptake of ¹⁵N per unit leaf area was 1.6- to 6-fold greater for 2-month-old leaves than for older leaves. The largest proportion of ¹⁵N remained in the treated leaf, although there was some acropetal movement to shoot tips. In a second experiment, 11.2 g N/liter (3.78% atom excess) urea- ¹⁵N and 3.4 g N/liter (4.92% atom excess) ¹⁵KNO₃ solutions of comparable osmotic potential were applied to 8-week-old leaves on 5-year-old 'Redblush' grapefruit field-grown trees of differing N status. Twenty-four percent of the applied ¹⁵N-urea was taken up after 1 hour and 54% after 48 hours. On average, only 3% and 8% of the K¹⁵NO₃ was taken up after 1 and 48 hours, respectively. Urea increased leaf N concentration by 2.2 mg N/g or 7.5% of total leaf N after 48 hours compared to a 0.5 mg N/g increase (1.8% of total leaf N) for KNO₃. Foliar uptake of ¹⁵N from urea, however, decreased ($P < 0.05$) with increasing total shoot N content after 48 hours ($r^2 = 0.57$).

Foliar N applications have been suggested as an alternative to conventional soil fertilization to reduce nitrate losses to groundwater systems (Council for Agricultural Science and Technology, 1985) and to reduce soil salinity (Embleton et al., 1978). Embleton and Jones (1974) presented evidence from eight field trials over 56 experiment years that showed that citrus production could be maintained by applying 3-6 foliar applications of urea per year, thus implying that 16%-33% of the annual requirement of citrus could be supplied with a single foliar application. Impey and Jones (1960) calculated that 70%-80% of a 5% (23.3 g N/liter) urea solution applied to 'Washington' navel leaves was absorbed by the abaxial surface of young and old leaves within 24 h. Neither of these studies used labeled N or accounted for any potential loss of N from the leaf surface or translocation of N out of leaves in that time.

Weinbaum (1978) estimated that a single application of foliar-applied NO₃ would provide only 0.7% of the total seasonal N demand of 2-year-old nonbearing prune trees, but the potential for increasing the concentration of KNO₃ in the spray solution is limited by the tolerance of the foliage to resist salt burn (Leece and Kenworthy, 1971). In olive, the redistribution of foliar-applied labeled ¹⁵N was increased by the high N sink demand of developing fruit and shoot tips (Klein and Weinbaum, 1984), but the N status of these small potted plants did not influence the subsequent absorption of foliar-applied urea. ¹⁵Nitrogen uptake from soil and the subsequent partitioning in 6-month-old citrus seedlings was strongly influenced by total N supply and the N demand for new growth, with a larger proportion of applied ¹⁵N taken up when N

supply was relatively low (Wallace, 1954; Lea-Cox, 1993). Since citrus roots have a greater N uptake-efficiency at low soil N concentrations, it follows that the same would be true for the foliar-uptake mechanism; i.e., the greatest concentration gradient exists from the surface into the leaf when leaf or shoot N is low. It is therefore hypothesized that foliar N uptake is likely to be more efficient when the demand for N is high, regardless of whether this demand is a function of low N or rapid growth. Since no quantitative data exist in the literature with respect to the uptake of foliar-applied ¹⁵N to citrus leaves from KNO₃ or urea-N, the objectives of this experiment were to describe this uptake from the two source materials over a 48-h period and to investigate whether this uptake was a function of whole-shoot N content.

Materials and Methods

Preliminary experiment. An initial phytotoxicity trial was conducted in which three weekly applications of 4.5, 9, 13.5, 18, and 22.4 g N/liter concentrations of low-biuret (<0.005 g biuret/liter) urea (Unocal Plus; Unocal Corp.) plus surfactant (0.1% v/v Triton X-77) were applied to leaves of 18-month-old field-grown 'Redblush' grapefruit trees on Swingle citrumelo (*C. paradisi* x *Poncirus trifoliata*) (SC) rootstock. Leaves (n = 8) of two age classifications (6-week- and 6-month-old) were hand-sprayed to runoff on individual shoots with each solution.

¹⁵Nitrogen application and uptake, potted tree (Expt. 1). Eight 18-month-old 'Redblush' grapefruit trees on SC, grown in full sun in 20-liter pots of Candler fine sand (Typic Quartsipsamments), were moved into the glasshouse before foliar application of material to avoid losses to dew or rainfall. The concentrated low-biuret urea solution (22.4 g N/liter) was diluted with distilled water and enriched with double-labeled ¹⁵N urea to give a 11.2 g N/liter urea solution with 1.776% atom excess ¹⁵N (Cabrera and Kisel, 1989). Shoots tagged at initiation on the individual trees were selected for the study. Five 2-month-old leaves on a single shoot on four individual trees and five 6-month-old leaves on a single shoot on another four trees were dipped into a beaker containing the ¹⁵N-urea solution for <5 sec. Any runoff from the leaves before drying was caught in the beaker. By weighing the beaker before and after dipping (to 0.001 g), the total weight of solution remaining on each leaf was recorded; leaves remained wet for ≈10 min. Glasshouse

Received for publication 27 May 1994. Accepted for publication 28 Nov. 1994. Florida Agricultural Experiment Station Journal Series no. R-03840. This research supported by the South-West Florida Water Management District under award no. 7273142-12. Additional support was provided by Vicksburg Chemical Company. 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 funding agencies, nor an endorsement of any registered product. J.D. Lea-Cox thanks the Univ. of Florida Foundation Herlong graduate assistant supplement for additional financial support. We thank Martin L. Smith, Jr. for his 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.

¹Current address: National Research Council Associate, NASA-Life Sciences, Mail Code MD-RES, Kennedy Space Center, FL 32899. To whom reprint requests should be addressed.

temperatures ranged from 20–32°C, the vapor pressure deficit (VPD) was 0–1.20 kPa, and maximum photon flux (PPF) was 1100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ over the 48-h experimental period. Two entire trees, one each with five 2- or 6-month-old treated leaves were harvested at 1.5, 6, 24, or 48 h after ^{15}N application to determine total leaf ^{15}N uptake and translocation to other plant parts. The treated leaves on each plant were surface-washed with distilled water at each harvest and their area was measured using a leaf area meter (LI-3000; LICOR, Lincoln, Neb.). These five leaves were then pooled into a single sample for ^{15}N analysis; the remaining leaves, stems, woody trunk, taproot, and fibrous roots were similarly pooled by tissue type and all samples were then dried for at least 48 h at 60°C. All tissue fractions were milled with a sample mill (Cyclotec 1093; Tecator, Sweden). Total N concentrations of each well-mixed tissue sample was 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, CW73VX, England) connected in series to the nitrogen analyzer determined the ^{14}N : ^{15}N ratio in each sample.

K^{15}NO_3 vs. ^{15}N -urea uptake and recovery, field trees (Expt. 2). A second ^{15}N -labeled experiment was conducted with three, 5-year-old ‘Redblush’ grapefruit trees on Volkamer lemon (*C. volkameriana* Ten. and Pasq.) (VL) and three trees on sour orange (*C. aurantium* L.) (SO) rootstock in the field. The objective was to determine the uptake of ^{15}N over four time periods (1.5, 6, 24, and 48 h) from two foliar N sources (KNO_3 , urea) and relate N uptake to total shoot N content. The six trees were selected on the basis of leaf N concentrations, which ranged from 21–27 mg N/g dry weight 3 months before ^{15}N application. Nitrogen concentrations of leaf and stem tissue were multiplied by their respective dry weights when harvested at the end of the experiment to derive an integrated value of total shoot N content, expressed in grams N per shoot. Eight terminal, non-fruiting, 8-week-old spring shoots were selected for uniformity at midpoint in the canopy of each of six trees in each cardinal direction, and were then randomly assigned to each treatment.

Since urea and KNO_3 have a similar salt index (73.6 vs. 75.4 units, respectively) (Rader et al., 1943), all leaves were treated with ^{15}N -urea and K^{15}NO_3 solutions of comparable osmotic concentration. The concentrations of N were 46.6% for urea and 13.9% for KNO_3 , so the salt index per unit N of KNO_3 was 3.29 times higher than that of urea. Accordingly, the final N concentration in the urea solution was 11.2 g N/liter (at 3.78% ^{15}N atom excess) and 3.4 g N/liter (at 4.92% ^{15}N atom excess) for KNO_3 . The third and fifth fully expanded leaves from the tip of each shoot, were tagged on each

tree for “N application during a clear dry period in March 1993. The fifth leaf was dipped in a solution of 0.1% (v/v) X-77 in distilled water for 5 sec, using the same technique as described for Expt. 1. These leaves were then harvested 24 h later to provide the background control percent ^{15}N for each treated shoot. Similarly, either ^{15}N -urea or K^{15}NO_3 was applied to the third leaf on each shoot after the control leaves had been harvested, and one shoot on each tree was harvested at 1, 6, 24, or 48 h later. Thus, there were six shoots harvested per N source at each harvest time, except in the urea 48-h treatment, where one shoot was broken and therefore discarded ($n=5$). The previous fall shoot leaves and stem behind each labeled spring shoot were also harvested and analyzed separately to determine whether any basipetal translocation had occurred out of the spring shoot within the uptake period. Night/day air temperatures were 14/27°C, the VPD was 2.1 kPa, and the maximum PPF was $=1300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ over the 48-h period.

At each harvest, shoots were placed in an ice chest and immediately transported to the laboratory. To avoid any potential loss of ^{15}N that may have been extracted from the leaf cuticle during washing with distilled water in Expt. 1, a cellulose acetate technique modified from Silcox and Holloway (1986) was used. Briefly, the treated leaves were carefully suspended by the petiole on a paper-clip and both leaf surfaces were sprayed with a 5% (w/v) cellulose acetate in acetone solution and allowed to dry in a fume hood. Upon drying, the cellulose acetate peeled away from the leaf surface and could be easily removed, such that no further cleaning of the leaf was necessary. The cellulose acetate was then carefully removed from each leaf and weighed to calculate remaining ^{15}N on the leaf surface at each time. The areas of the treated leaf and shoot leaves and their dry weights were measured. The treated leaves, shoot tissue fractions, and cellulose acetate peelings were separately milled and analyzed for N and ^{15}N as in Expt. 1. The N uptake data were tested for significant differences using a two N source $\times$ four uptake time repeated measures analysis of variance with six replicates (Littel, 1989). 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).

Results

Preliminary experiment. No foliar burn was noted on either 6-week- or 6-month-old leaves at 4.5 and 9 g N/liter after 3 weeks. Foliar burn of the young leaves was evident after one spray of 18 or 22.4 g N/liter urea and after two sprays of 13.5 g N/liter urea, but the old leaves were undamaged even after a third weekly 13.5 g N/

Table 1, Total plant ^{15}N uptake, applied ^{15}N per unit leaf area, and percentage uptake of ^{15}N by young (6-week-old) and old (6-month-old) sun-adapted ‘Redblush’ grapefruit leaves 1.5, 6, 24, and 48 h after leaves were dipped in a solution of ^{15}N -urea in a glasshouse. Each value = Σ (pooled ^{15}N atom excess for all tissues)/treatment leaf area (single-side).

Treatment	^{15}N uptake/ unit leaf area ($\mu\text{g}\cdot\text{cm}^{-2}$)	^{15}N applied/ unit leaf area ($\mu\text{g}\cdot\text{cm}^{-2}$)	^{15}N uptake (% of applied)
Young, 1.5 h	0.041	0.428	9.6
Old, 1.5 h	0.012	0.305	3.9
Young, 6.0 h	0.138	0.323	42.7
Old, 6.0 h	0.022	0.320	6.9
Young, 24 h	0.048	0.219	22.0
Old, 24 h	0.059	0.433	13.6
Young, 48 h	0.038	0.234	16.3
Old, 48 h	0.031	0.306	10.1

^aWhole shoot dipped.

liter spray. In contrast, most 6-month-old leaves abscised after one spray of 18 or 22.4 g N/liter urea. Thus, subsequent experiments used a 11.2 g N/liter concentration of urea that could be safely applied to all leaves. No foliar burn symptoms were noted at any time in Expts. 1 and 2.

Experiment 1. Total ^{15}N uptake at each time (Table 1) was expressed as the sum of ^{15}N atom excess values (mg ^{15}N) from all pooled tissue samples (analyzed separately), normalized on a one-sided leaf surface area basis. The total percentage uptake of ^{15}N by young, 2-month-old leaves was 1.6 to 6 times greater at each harvest time than that from the older 6-month-old leaves (Table 1). The young leaves used for the 6-h harvest were relatively small so the whole terminal shoot was dipped in the solution, which apparently greatly increased the percentage uptake into this shoot. Uptake per unit leaf area of this particular treatment was normalized by including the stem area of the shoot (Table 1), but the uptake of this treatment was still larger than other treatments. The largest proportion of the recovered ^{15}N was found in the pooled treated leaf sample on each shoot, but translocation of ^{15}N out of the treated shoot into other plant parts after 24 h totaled 15% and 38% from the young and older leaf treatments, respectively (data not shown). These amounts did not change over the subsequent 24-h period, with an average of 15% and 40% translocated from the young and old leaves, respectively, after 48 h. The greatest propor-

tion (51 %–78 %) of this translocated ^{15}N was found to be in other young shoots on the plant from the tissue analysis, indicating acropetal shoot movement of the translocated ^{15}N , irrespective of the age of the treated leaves. There was some concern expressed that ^{15}N may have been extracted from the leaf cuticle during the washing with distilled water after harvest in Expt. 1, and so the cellulose acetate spray technique was subsequently used in Expt. 2 to remove leaf surface deposits.

Experiment 2. Total ^{15}N -urea uptake by the treated leaf after 1 and 48 h was 24% and 54%, compared to only 3% and 7.5% recovered from the KNO_3 -treated leaf at the same times (Fig. 1). Total percentage recoveries in the shoots ranged from 2%–8% for urea-treated shoots and from 1%–3% in KNO_3 -treated shoots over the 48 h period. The ^{15}N recovered from the cellulose acetate peelings after 1 h averaged 11% of the applied ^{15}N -urea and 45% of the K^{15}NO_3 . The uptake of ^{15}N -urea decreased after 24 h ($P < 0.10$) and 48 h ($P < 0.05$), with increasing total N content of shoots (Fig. 2). There was no relationship between K^{15}NO_3 uptake and N content of the shoot.

Discussion

The total concentration of N in the urea-N solution was 3.29 times (11.2/3.4) greater than that in the KNO_3 solution, which

Fig. 1. ^{15}N uptake per unit treated leaf area ($\mu\text{g cm}^{-2}$) from vegetative spring shoots on field-grown ($n = 6$) trees, 1, 6, 24 and 48 h after foliar applications of 11.2 g N/liter urea and 3.4 g N/liter KNO_3 solutions (of equal osmotic potential). Percentage values are the mean ($n = 6$) ^{15}N recovered after 48 h. Error bars represent 1 se from the mean.

contributed to greater uptake of urea-N over the 48-h uptake period. Despite the disparity in solution N concentrations, it appeared that the overall uptake of N from urea was greater than that from KNO_3 . Assuming a linear relationship between N concentration applied to the leaf and N uptake rate, the application of an equivalent N concentration from KNO_3 would have given $\approx 8.1\% \times 3.29$, or 26.6%, compared to the 57.4% taken up from urea (Fig. 1). Since the cuticle is nonpolar, movement of ionic compounds (like KNO_3) through the cuticle might be expected to be less than that of a polar, but nonionic compound like urea. Since diffusion also depends on the volatility of a compound, the volatility of urea in the cuticle, although low, is likely to be greater than that of KNO_3 (P. Petracek, Dept. of Citrus, Lake Alfred, personal communication).

It is possible that three separate KNO_3 applications would have been a better comparison to one application of urea with the additional K benefit of the KNO_3 treatment. The total uptake of ^{15}N over the 48-h period in this experiment was slightly lower than the uptake calculated by Impey and Jones (1960), whose studies used washings of adjacent leaves to calculate N uptake by difference from that applied. The disparity in uptake rates between Expts. 1 and 2 in this study maybe related in large part to the leaf washing technique used in the preparation of leaves for analysis. In the first experiment, leaves were washed thoroughly in distilled water to remove all surfactant and urea residues. It was possible that ^{15}N

within the loose structure of the leaf cuticle, but not yet metabolized by leaf cells, could have been leached from the cuticle by this washing with distilled water. In addition, any ^{15}N adhering to the cuticle surface may have not been removed. The cellulose acetate technique used in the field experiment, which removed the outer cuticle layer, dirt and any surface residues, but not the inner cuticle layers, intended to resolve both problems.

To investigate whether foliar N applications can supply a significant proportion of total leaf N requirements, the mean total N uptake over the 48 h was calculated as $\text{mg } ^{15}\text{N}$ divided by the ^{15}N enrichment of solution = total N from ^{15}N applied, divided by the mean total N content of the treated leaves, (e.g., $46.25 \text{ mg} \times 0.0378 = 1.223 \text{ mg } [^{14}\text{N} + ^{15}\text{N}]/16.2 \text{ mg N}$). Thus, 7.6% of the total N content in leaves came from the single application of urea and 1.7% came from KNO_3 after 48 h. This was greater than the 0.7% N from a 1.2%-N KNO_3 application to prune trees (Weinbaum, 1978). Since the mean N concentration of the urea-treated leaves was 29.3 and 27.3 $\text{mg} \cdot \text{g}^{-1}$ for KNO_3 -treated leaves, this would equate to an increase in leaf N concentration of 2.2 or 0.5 $\text{mg} \cdot \text{g}^{-1}$ from each respective N source. The negative relationship between the vegetative N content of shoots and ^{15}N -urea uptake (Fig. 2) indicated that the shoots with lower total N content absorbed ^{15}N -urea more rapidly up to 48 h after application than shoots with higher N content. Thus, relatively high concentration gradients between N on the outside and inside shoots, tended to enhance ^{15}N uptake. The

Fig. 2. ^{15}N uptake per unit treated leaf area ($\mu\text{g} \cdot \text{cm}^{-2}$) vs. whole shoot N content (g) 24 and 48 h after foliar applications of 11.2 g N/liter urea and 3.4 g N/liter KNO_3 solutions (of equal osmotic potential) to shoots on 5-year-old 'Redblush' grapefruit trees in the field.

total amount of $K^{15}NO_3$ absorbed over the experimental time course was not large enough to be affected by total shoot N content. Widders (1991) found that >40% of foliar-absorbed ^{15}N -urea applied to a single fully expanded tomato (*Lycopersicon esculentum* L. 'Saladette') leaf was translocated to nontreated plant (primarily fruit) tissue within 7 days, such that single foliar applications of N did not significantly alter the total N concentrations in tomato leaves. The maintenance of a large N concentration differential by growing tissues is therefore likely to enhance the uptake of foliar-applied N.

In summary, it appears that foliar applications of urea to *Citrus* can significantly increase leaf N concentrations within 48 h of application, but repeated applications may be subject to decreased rates of uptake by increasing shoot N status. These data do not contradict the conclusions of Embleton and Jones (1974) that six foliar applications of urea per year may be sufficient to maintain the production of bearing citrus trees. More experimentation will be required to determine if an increase of $1 \mu g \cdot cm^{-2}$ per spray or 7.6% of the total N is sufficient to maintain optimum leaf N concentration over the long term. It would be interesting to repeat this study to include fruiting and nonfruiting shoots to determine whether additional fruit sinks increase the efficiency of foliar-N uptake. If this were true, the timing of foliar N applications would become critical in maximizing N uptake efficiency. Future work could also investigate the uptake efficiency and timing of repeated foliar urea-N applications to the same shoot and whether the increased salt load of repeated applications of KNO_3 on leaves has additional physiological implications with regard to N and K uptake.

Literature Cited

- Cabrera, M.L. and D.E. Kissel. 1989. Review and simplification of calculations in ^{15}N tracer studies. *Fert. Res.* 20:11-15.
- Council for Agricultural Science and Technology. 1985. Agriculture and ground water quality. Council Agr. Sci. Technol. Rpt. 103.
- Embleton, T.W. and W.W. Jones. 1974. Foliar-applied nitrogen for citrus fertilization. *J. Environ. Qual.* 3:388-391.
- Embleton, T.W., M. Matsumura, L.H. Stolzy, D.A. Devitt, W.W. Jones, R. El-Motaium, and L.L. Summers. 1978. Citrus nitrogen fertilizer management, groundwater pollution, soil salinity and nitrogen balance. *Applied Agr. Res.* 1(1):57-64.
- Impey, R.L. and W.W. Jones. 1960. Rate of absorption of urea by intact leaves of Washington navel orange. *Proc. Amer. Soc. Hort. Sci.* 76:181-185.
- Klein, I. and S.A. Weinbaum. 1984. Foliar application of urea to olive: Translocation of urea nitrogen as influenced by sink demand and nitrogen deficiency. *J. Amer. Soc. Hort. Sci.* 109:356-360.
- Lea-Cox, J.D. 1993. Nitrogen uptake, nitrogen-use efficiency, and nitrogen leaching losses of citrus. PhD diss. Univ. of Florida, Gainesville.
- Leece, D.R. and A.L. Kenworthy. 1971. Effects of potassium nitrate foliar sprays on leaf nitrogen concentration and growth of peach trees. *HortScience* 6:171-173.
- Littel, R.C. 1989. Statistical analysis of experiments with repeated measurements. *HortScience* 24:37-40.
- Montgomery, D.C. 1984. Design and analysis of experiments, 2nd ed. Wiley, New York. p. 189-201.
- Rader, Jr., L.F., L.M. White, and C.W. Whittaker. 1943. The salt index—A measure of the effect of fertilizers on the concentration of the soil solution. *Soil Sci.* 55:201-218.
- Silcox, D. and P.J. Holloway. 1986. A simple method for the removal and assessment of foliar deposits of agrochemicals using cellulose acetate film stripping. *Aspects of Applied Biol.* 11:13-17.
- Wallace, A. 1954. Ammonium and nitrate nitrogen absorption by citrus. *Soil Sci.* 78:89-94.
- Weinbaum, S.A. 1978. Feasibility of satisfying total nitrogen requirement of non-bearing prune tree with foliar nitrate. *HortScience* 13:52-53.
- Widders, I.E. 1991. Absorption and translocation of foliar applied triazone-N as compared to other nitrogen sources in tomato. *J. Plant Nutr.* 14: 1035-1045.