Nitrogen Uptake by Citrus Leaves

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Abstract. We studied whether foliar-applied N uptake from a single application of low-biuret 'N-urea or K'NO3 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 'N-urea) solution with 0.1% (v/v) Triton X-77. Two entire trees were harvested 1.5, 6, 24, and 48 hours after 'N application. Uptake of 'N per unit leaf area was 1.6- to 6-fold greater for 2-month-old leaves than for older leaves. The largest proportion of 'N 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) K'NO3 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 'N-urea was taken up after 1 hour and 54% after 48 hours. On average, only 3% and 8% of the K'NO3 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 K'NO3. Foliar uptake of 'N from urea, however, decreased (P < 0.05) with increasing total shoot N content after 48 hours (r² = 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, 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 'N to citrus leaves from K'NO3 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, and 18.24 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.

'Nitrogen application and uptake, potted tree (Exp. 1). Eight 18-month-old 'Redblush' grapefruit trees on SC, grown in full sun in 20-liter pots of Candler fine sand (Typic Quartzipsamments), were moved into the glasshouse before foliar application of material to avoid losses to dew or rainfall. The concentrated low-biuret urea solution (224.3 g N/liter) was diluted with distilled water and enriched with double-labeled 'N urea to give a 11.2 g N/liter urea solution with 1.776% atom excess 'N (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 'N-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
temperatures ranged from 20–32°C, the vapor pressure deficit (VPD) was 0.1–20 kPa, and maximum photon flux (PPF) was 1100 μmol·m⁻²·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 ¹⁵N application to determine total leaf ¹⁵N 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; LI-COR, Lincoln, Neb.). These five leaves were then pooled into a single sample for ¹⁵N 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 ¹⁵N:¹⁴N ratio in each sample.

K¹⁵NO₃ vs. ¹⁴N-urea uptake and recovery, field trees (Expt. 2). A second ¹⁵N-labeled experiment was conducted with three, 5-year-old ‘Redblush’ grapefruit trees on Volkamer lemon (Citrus 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 ¹⁵N over four time periods (1.5, 6, 24, and 48 h) from two foliar N sources (KNO₃, urea) and relate N uptake to total shoot N content. The six trees were selected for uniformity at midpoint in the canopy of each of six trees and their area was measured using a leaf area meter (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 ¹⁵N:¹⁴N ratio in each sample.

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 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 ¹⁵N uptake, applied ¹⁵N per unit leaf area, and percentage uptake of ¹⁵N 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 ¹⁵N-urea in a glasshouse. Each value = Σ (pooled ¹⁵N atom excess for all tissues)/treatment leaf area (single-side).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>¹⁵N uptake/ unit leaf area (µg·cm⁻²)</th>
<th>¹⁵N applied/ unit leaf area (µg·cm⁻²)</th>
<th>¹⁵N uptake (% of applied)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young, 1.5 h</td>
<td>0.041</td>
<td>0.428</td>
<td>9.6</td>
</tr>
<tr>
<td>Old, 1.5 h</td>
<td>0.012</td>
<td>0.305</td>
<td>3.9</td>
</tr>
<tr>
<td>Young, 6.0 h</td>
<td>0.138</td>
<td>0.323</td>
<td>42.7</td>
</tr>
<tr>
<td>Old, 6.0 h</td>
<td>0.022</td>
<td>0.320</td>
<td>6.9</td>
</tr>
<tr>
<td>Young, 24 h</td>
<td>0.048</td>
<td>0.219</td>
<td>22.0</td>
</tr>
<tr>
<td>Old, 24 h</td>
<td>0.059</td>
<td>0.433</td>
<td>13.6</td>
</tr>
<tr>
<td>Young, 48 h</td>
<td>0.038</td>
<td>0.234</td>
<td>16.3</td>
</tr>
<tr>
<td>Old, 48 h</td>
<td>0.031</td>
<td>0.306</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Whole 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 *¹⁵N* uptake at each time (Table 1) was expressed as the sum of *¹⁵N* atom excess values (mg *¹⁵N*) from all pooled tissue samples (analyzed separately), normalized on a one-sided leaf surface area basis. The total percentage uptake of *¹⁵N* 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 *¹⁵N* was found in the pooled treated leaf sample on each shoot, but translocation of *¹⁵N* 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 proportion (51%–78%) of this translocated *¹⁵N* was found to be in other young shoots on the plant from the tissue analysis, indicating acropetal shoot movement of the translocated *¹⁵N*, irrespective of the age of the treated leaves. There was some concern expressed that *¹⁵N* 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 *¹⁵N*-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 KNO₃-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 KNO₃-treated shoots over the 48 h period. The *¹⁵N* recovered from the cellulose acetate peelings after 1 h averaged 11% of the applied *¹⁵N*-urea and 45% of the KNO₃. The uptake of *¹⁵N*-urea decreased after 24 h (% < 0.10) and 48 h (% < 0.05), with increasing total N content of shoots (Fig. 2). There was no relationship between KNO₃ 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 KNO₃ solution, which

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Fig. 1. *¹⁵N*-nitrogen uptake per unit treated leaf area (µg·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 KNO₃ solutions (of equal osmotic potential). Percentage values are the mean (n=6) *¹⁵N* 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 KNO₃. Assuming a linear relationship between N concentration applied to the leaf and N uptake rate, the application of an equivalent N concentration from KNO₃ would have given ≈8.1% × 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 KNO₃) 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 volubility of a compound, the volubility of urea in the cuticle, although low, is likely to be greater than that of KNO₃ (P. Petracek, Dept. of Citrus, Lake Alfred, personal communication).

It is possible that three separate KNO₃ applications would have been a better comparison to one application of urea with the additional K benefit of the KNO₃ treatment. The total uptake of ¹⁵N 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 ¹⁵N 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 ¹⁵N 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 mg ¹⁵N divided by the ¹⁵N enrichment of solution = total N from ¹⁵N applied, divided by the mean total N content of the treated leaves, (e.g., 46.25 mg × 0.0378 = 1.223 mg [¹⁵N/¹⁴N]/16.2 mg N). Thus, 7.6% of the total N content in leaves came from the single application of urea and 1.7% came from KNO₃ after 48 h. This was greater than the 0.7% N from a 1.2%-N KNO₃ application to prune trees (Weinbaum, 1978). Since the mean N concentration of the urea-treated leaves was 29.3 and 27.3 mg·g⁻¹ for KNO₃-treated leaves, this would equate to an increase in leaf N concentration of 2.2 or 0.5 mg·g⁻¹ from each respective N source. The negative relationship between the vegetative N content of shoots and ¹⁵N-urea uptake (Fig. 2) indicated that the shoots with lower total N content absorbed ¹⁵N-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 ¹⁵N uptake. The

Fig. 2. ¹⁵Nitrogen uptake per unit treated leaf area (µg·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 KNO₃ solutions (of equal osmotic potential) to shoots on 5-year-old 'Redblush' grapefruit trees in the field.
total amount of K NO 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 15N-urea applied to a single fully expanded tomato (Lycopersicon esculentum L. ‘Saladete’) 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 µg·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 KNO₃ on leaves has additional physiological implications with regard to N and K uptake.

Literature Cited