The Influence of Foliar-applied Urea Nitrogen and Saline Solutions on Net Gas Exchange of *Citrus* Leaves

R. Romero-Aranda and J.P. Syvertsen

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

Additional index words. NaCl, CaCl₂, net CO₂ assimilation, stomatal conductance, water use efficiency, photosynthetic nitrogen use efficiency, *Citrus paradisi* 'Duncan' grapefruit, *Citrus sinensis* 'Valencia' orange

Abstract. We compared net gas exchange rates of CO₂ and H₂O vapor of greenhouse-grown 'Duncan' grapefruit (*Citrus paradisi* Macf.) and 'Valencia' orange [*C. sinensis* (L.) Osbeck] leaves after multiple foliar sprays of urea N with and without NaCl : CaCl₂ solutions. Highly saline solutions (3.8 dSm⁻¹) caused necrotic burn symptoms after leaf chloride levels reached 7 mmol·m⁻². Grapefruit leaves had higher leaf Cl and more burn symptoms than orange leaves. The remaining green areas of all salt-stressed leaves, however, had similar rates of net CO₂ assimilation (A_{CO2}) and stomatal conductance (gs) as water-sprayed control leaves. Total leaf N and chlorophyll increased with repeated foliar applications of urea solutions regardless of salinity levels in the spray solution. Thus, salts in solution did not interfere with foliar absorption of N. High urea N solutions (33.6 g·liter⁻¹) without salts caused foliar burn and leaf abscission after one application. Three sprays of urea-N solution (11.2 g·liter⁻¹) increased N concentration of N-deficient leaves about 60% and increased A_{CO2} rate about 50%. A_{CO2} did not increase when nitrogen concentration in leaves exceeded a threshold value of about 200 mmol·m⁻² so photosynthetic nitrogen use efficiency (PNUE = A_{CO2}/N) decreased with increasing leaf N concentration. Net gas exchange and PNUE was higher for grapefruit than for orange leaves. Leaf Cl levels from foliar-applied salts may not be as detrimental to leaf gas exchange as Cl from salts in soil-applied irrigation water.

Nitrogen availability limits plant growth and productivity by limiting leaf area growth and/or photosynthetic capacity (Sinclair, 1990). When leaf N concentration is low, net CO₂ assimilation rate (A_{CO2}) increases with additional N (Evans, 1989; Syvertsen, 1987). Application of fertilizer N directly to leaves, especially urea N, can be a potential alternative to conventional soil fertilization without contributing to soil salinity and potential groundwater contamination (Embleton et al., 1986). Urea sprays can cause leaf burn, however, when applied at high concentrations or sprayed on young leaves (Lea-Cox and Syvertsen, 1995). The success of supplying annual nitrogen requirements of citrus trees using urea depends on adequate amounts of N penetrating into leaves while avoiding phytotoxicity. Citrus leaves take up urea N more rapidly than potassium nitrate N partly because of the lower osmotic potentials of KNO₃ solution (Lea-Cox and Syvertsen, 1995), and also because nonpolar urea readily diffuses through the cuticle (Wittwer et al., 1963). Like many other crop plants, citrus leaves are sensitive to salt injury when saline solutions come into contact with leaves (Mass and Hoffman, 1977).

Adverse effects of soil salinity on citrus growth and fruit yield are well documented (Maas, 1993; Shalhevet and Levy, 1990). High foliar Cl concentration from saline soil can reduce A_{CO2} and stomatal conductance (gs) (Lloyd and Howie, 1989). The impact of high Cl may be determined, in part, by the ability of the rootstock or scion to exclude Cl (Behboudian et al., 1986). Nonstomatal factors can also reduce A_{CO2} due to Na and Cl toxicity effects (Lloyd et al., 1987). Saline coastal fog (Maas, 1993) and saline irrigation water (Eaton and Harding, 1959) can be phytotoxic. Citrus leaves can also accumulate Cl and Na when agricultural spray solutions are prepared with saline water (Syvertsen et al., 1989). Urea also may be included as an adjuvant in spray solutions

to improve the effectiveness of foliar-applied chemicals (Beckett and Stoller, 1991; Kannan, 1980). Potential effects of foliar-applied urea N and salts on citrus leaf gas exchange have not been described.

The purpose of these studies was to determine uptake and potential phytotoxicity of citrus leaves sprayed with foliar urea N and chlorides. We varied the previous nutritional status of the leaves and studied the relationships between net gas exchange, Cl, N, and chlorophyll concentration in leaves of 'Duncan' grapefruit and 'Valencia' orange. We hypothesized that foliar-applied urea N and salts would interact to affect their penetration and net gas exchange of citrus leaves.

Material and Methods

We designed two greenhouse experiments to determine levels of foliar uptake and phytotoxicity of Cl and urea on net gas exchange responses of leaves. The first experiment was designed to study well-nourished leaf responses to a broad range of salt, urea, and urea plus salt concentrations in foliar-applied solutions. The second experiment used N deficient seedlings and tested leaf responses to an intermediate urea concentration with a relatively high salinity concentration in the spray solution.

Plant material. One-year-old seedlings of 'Duncan' grapefruit and 'Valencia' orange were selected for uniformity and transplanted from sand into 2.3-liter pots of a commercial soil medium of 3 peat : 1 perlite : 3 vermiculite : 3 pine bark (by volume, with added N and P) (V.J. Growers, Apopka, Fla.) Seedlings were grown in a greenhouse under 50% shade, irrigated every other day, and fertilized weekly with Hoagland's solution. When seedlings were 14 months old, all plants were trained to a single shoot.

Experiment 1. 24 uniform 'Duncan' grapefruit and 24 'Valencia' orange seedlings were selected for foliar spray applications of 2 levels of saline (NaCl:CaCl₂, 3:1) solution, 3 levels of low-biuret urea, 224 g N/liter (Unocal Plus; Unocal, West Sacramento, Calif.), 2 combinations of salt + urea, and a water-sprayed control (Table 1). All spray solutions contained 0.5 % v/v Triton X-77 as a surfactant. Each of the eight spray treatments was applied weekly

Florida Agricultural Experiment Station journal series no. R-4634. We thank the Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA, Spain) for the fellowship provided to R.R.-A. and support from FCPAC (no. 927-6) to J.P.S. during the course of this study. We also thank Martin L. Smith, Jr. and T. Gater for their 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.

Table 1. Electrical conductivity (EC) and urea N concentration of foliar applied solutions on young (expanding) and mature leaves of 'Duncan' grapefruit and 'Valencia' orange seedlings in Expts. 1 and 2.

Sprays treatments	Expt. 1		Expt. 2	
	EC (dS·m ⁻¹)	N (g·liter ⁻¹)	EC (dS·m ⁻¹)	N (g·liter ⁻¹)
Control	0.06 ^z	---	0.24 ^y	---
Salt				
Low	0.51	---	---	---
Intermediate (IS)	3.76	---	---	---
Urea				
Low	0.52	6.72	---	---
Intermediate (IU)	1.02	13.44 ^x	1.18	11.20 ^w
High (HU)	2.45	33.60	---	---
Salt + urea				
IS + IU	3.79	13.44	---	---
IS + HU	3.79	33.60	---	---
HS + IU	---	---	9.31	11.20

^zDeionized water + surfactant.

^yTap water + surfactant.

^x1.34% Urea-N, 1.34%.

^w1.12% Urea-N, 1.12%.

to 3 seedlings beginning in June 1994, when plants were 15 months old. Seedlings were sprayed to runoff but the soil surface was covered with a plastic film until leaves dried to prevent any soil deposition. During the 11-week period, mean maximum/minimum air temperatures in the greenhouse were 41(±3)/25(±1) C and mean maximum air vapor pressure deficit (VPD) averaged 5.0 (±1.3) kPa. During week 5, leaf N concentration in some treatments exceeded 300 mmol·m⁻² (about 5% dry weight), which is considered excessively high for citrus leaves in the field (Hanlon et al., 1995). In week 6, pots were thoroughly leached with tap water and soil fertilization was stopped to allow leaf nitrogen concentration to decrease.

Experiment 2. Well-nourished 14-month-old 'Duncan' grapefruit and 'Valencia' orange seedlings were allowed to become nitrogen-deficient by not fertilizing for 2 months. After that period, total nitrogen concentration in leaves was from 93 to 135 mmol·m⁻², and there was some visible yellowing. Twelve uniform seedlings of each species were selected for foliar applications of urea + saline solutions. We studied the effects of a high saline spray (EC = 9.31 dS·m⁻¹) with and without an intermediate urea concentration (11.2 g N/liter, Table 1). Three seedlings were sprayed weekly to runoff while preventing any soil deposition as described for Expt. 1. Spray treatments began in October 1994 when seedlings were 14 months old. During the 6-week period, mean maximum/minimum air temperatures were 37(±3)/22(±2) C, and mean maximum daytime VPD averaged 4.1 (±1.2) kPa.

Gas exchange measurements. In both experiments, we measured A_{CO2} and gs with a LI-6200 portable photosynthesis system (Li-Cor, Lincoln, Neb.) using a 250cm³ cuvette. All measurements were made in the morning (0830–0930 HR) to avoid high afternoon temperatures and VPD. Gas exchange measurements were made on 'Valencia' orange in Expt. 1 every 2–3 weeks. All other measurements were made weekly on 2 fully expanded, mature leaves (≈ 3 months old) on each seedling, and were repeated using the same leaves whenever possible. When burn symptoms were visible, measurements were made only on the green-appearing remaining leaf surfaces by placing the measurement cuvette over the green area only. Young, fully expanded, but not mature leaves about 1 month old, were also measured during week 12 at the end

Table 2. Leaf chloride (Cl) and nitrogen concentration (N) on 'Duncan' grapefruit mature leaves after 10 weeks of foliar treatments described for Expt.1 in Table 1.

Spray treatments	Cl (mmol·m ⁻²)	N (mmol·m ⁻²)
Control	0.81 ± 0.07	124 ± 6
Salt		
Low	1.72 ± 0.05	114 ± 8
Intermediate (IS)	8.63 ± 0.08	127 ± 11
Urea		
Low	0.71 ± 0.06	265 ± 15
Intermediate (IU)	0.82 ± 0.04	353 ± 20
High (HU)	---	---
Salt + urea		
IS + IU	5.61 ± 0.16	297 ± 23
IS + HU	---	---

^zNo mature leaves remained on the seedlings after 10 weeks of this treatment.

of Expt. 1. During all measurements in Expt. 1, radiation within the cuvette was supplemented with a 300 W tungsten-halogen lamp (Sylvania, ELH, Winchester, Ky.) after cooling by filtering through 2 cm of circulating cold water. All measurements were made at photosynthetic photon flux (PPF, 400–700 nm) of 1000–1200 μmol·m⁻²·s⁻¹, which exceeds saturating PPF for citrus, (Syvertsen, 1984), leaf temperatures of 32 ± 2C and leaf to air VPD of 2.4 ± 0.4 kPa within the cuvette. In Expt. 2, all measurements were made while supplementing PPF with a Qbeam™ solid state LED lighting system (Quantum Devices, Barneveld, Wis.) (Tennessen et al., 1994) because it did not require any cooling. During these measurements, the PPF was set at 850 μmol·m⁻²·s⁻¹ at 670 nm, average leaf temperature was 26 ± 3C and leaf to VPD was 1.4 ± 0.5 KPa within the cuvette.

Foliar analysis. All leaves were rated visually for percentage of necrotic area. To avoid removing too many leaves for analysis, only one leaf of the same age class as those used for gas exchange measurements was removed from each seedling every 2–3 weeks in Expt. 1. In Expt. 2, two leaves per seedling were removed every week for analysis. Leaf fresh weight and leaf area (LI-3000; LI-COR) were determined; leaves were quickly rinsed with distilled water and blotted dry. For chlorophyll analysis, two 9 mm diameter discs were punched from green area near the mid-laminar region of each leaf and extracted with N,N dimethylformamide for at least 72 h in the dark at 4C. Absorbance was determined using a spectrophotometer (Ultrospec II; LKB Biochrom, Cambridge, U.K.) at 647 and 664 nm and used to calculate total chlorophyll concentrations (Chl) (Porra et al., 1989). An index of leaf succulence was calculated as (fresh weight-dry weight) divided by leaf area (Gratani, 1993). Entire leaves, minus the 2 discs but including any necrotic tissue, were oven dried for at least 72 h at 60C and ground to a powder before total nitrogen (semimicro Kjeldahl) and chloride (HBI Chloridometer; Haake Buchler, Saddle Brook, N.J.) analyses.

Data were analyzed for significant differences ($P < 0.05$) using a factorial analysis of variance with species, spray solution, and number of weekly sprays as main factors. Relationships between leaf N concentration and gas exchange characteristics were described using linear or quadratic regression analyses where appropriate.

Results

Experiment 1. After 11 weekly sprays, leaf chloride concentration increased with the salt concentration of the spray solution

Fig. 1. Net CO_2 assimilation rate (A_{CO_2}) (A) and stomatal conductance (g_s) (B) of 'Duncan' grapefruit mature leaves in response to weekly foliar sprays of NaCl-CaCl_2 solution ($3.76 \text{ dS}\cdot\text{m}^{-1}$) (●) or water-sprayed control (○) in Expt. 1. Each data point is the mean of $n = 6$ leaf measurements; vertical bars indicate $\pm \text{SD}$.

(Table 2). There were no visible necrotic burn symptoms on 'Valencia' leaves. There were insufficient leaves for replicate CI and N samples from 'Valencia' orange so only replicated data for 'Duncan' grapefruit are shown. Burn symptoms on grapefruit leaf margins and tips appeared when CI levels exceeded $7 \text{ mmol}\cdot\text{m}^{-2}$. Mean CI concentrations of the young leaves from both species developed during spray treatments were $0.9 \text{ mmol}\cdot\text{m}^{-2}$ for the low and $5.9 \text{ mmol}\cdot\text{m}^{-2}$ for the intermediate saline treatments.

Grapefruit leaves sprayed with the low and intermediate urea concentration had two-fold and three-fold the N concentration of control leaves (Table 2). Young leaves sprayed with the high urea concentration developed burn symptoms as they expanded and subsequently abscised. Sprays of high urea (33.6 g N/liter) caused necrotic spots over 10% of grapefruit leaf surfaces after only one spray. Leaves sprayed 3 times with intermediate salt plus high urea were 80%–90% necrotic and since they subsequently abscised, were not analysed. Mature leaves sprayed with low and intermediate concentration of urea without salt were not visibly injured after 10 weekly sprays. Individual leaf area and leaf succulence index were not affected by treatment or species tested. At the end of the experiment, mean individual leaf area was $22.0 \pm 4.4 \text{ cm}^2$ for mature leaves, and $27.3 \pm 6.2 \text{ cm}^2$ for fully expanded young leaves. The leaf succulence index was $17.3 \pm 4.5 \text{ mg}\cdot\text{cm}^{-2}$ for mature leaves and $19.5 \pm 3.6 \text{ mg}\cdot\text{cm}^{-2}$ for fully expanded young leaves.

Net A_{CO_2} and g_s of 'Duncan' grapefruit leaves were not significantly affected ($P > 0.05$) by salinity sprays (Fig. 1). The gradual

Table 3. Regression coefficients (β_n) and coefficient of determination (r^2) for relationships between dry weight per leaf area (DW/a), CO_2 assimilation rate (A_{CO_2}), water use efficiency (WUE), and photosynthetic N use efficiency (PNUE)(y) and the leaf N concentration (x) of mature leaves and young leaves of 'Duncan' grapefruit. Data from Expt. 1, Fig. 2.

y vs. x	Leaf age	β_0	β_1 (x)	β_2 (x^2)	r^2
DW/a vs. N	Mature	69.43	0.04	---	0.40
	Young	40.26	0.19	---	0.74
A_{CO_2} vs. N	Mature	4.12	0.08	-1.6×10^{-4}	0.5
	Young	3.25	0.05	---	0.87
WUE vs. N	Mature	2.06	0.01	-1.7×10^{-5}	0.23
	Young	2.36	0.02	---	0.87
PNUE vs. N	Mature	0.12	-2.4×10^{-4}	---	0.85
	Young	0.10	-2.3×10^{-4}	---	0.64

Fig. 2. The relationship of dry weight per unit leaf area (DW/a) (A), net CO_2 assimilation rate (A_{CO_2}) (B), water use efficiency (WUE)(C), and photosynthetic N use efficiency (PNUE)(D) to the leaf N concentration ($\text{mmol}\cdot\text{m}^{-2}$) of mature (○) and young leaves (●) of 'Duncan' grapefruit in Expt. 1. Each data point is the mean of 6 leaf measurements. Regression equations and the coefficients of determination are presented in Table 2.

decrease in A_{CO_2} from about 15 to $12 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during the 10 week experimental period was probably due to leaf aging. Stomatal conductance, however, did not decrease as leaves aged. Gas exchange data from 'Duncan' grapefruit were very similar to those from 'Valencia' orange leaves (data not shown).

Leaf N increased with the concentration of N in urea sprays and also increased linearly with the number of weekly sprays (data not shown). To investigate relationships between leaf N and leaf characteristics, all data from mature and young leaves were pooled at the end of the experiment without regard to urea spray treatment. Dry weight per leaf area (DW/a), A_{CO_2} , and water use efficiency ($\text{WUE} = A_{\text{CO}_2}/\text{transpiration}$) were all positively correlated (Table 3, Fig. 2) with the leaf N of young leaves. For mature leaves, there was no increase in dry weight per leaf area or WUE as leaf N increased (Table 3, Fig. 2). There was little effect of leaf N on A_{CO_2}

Fig. 3. The effect of weekly foliar sprays of NaCl-CaCl₂ solution (EC = 9.31 dS·m⁻¹) on mature leaf chloride (Cl) concentration on 'Duncan' grapefruit (●) ($y = 1638x + 509$, $r^2 = 0.98$) and 'Valencia' orange (■) ($y = 867x + 567$, $r^2 = 0.96$) in Expt. 2. Water-sprayed control EC = 0.24 dS·m⁻¹ (○, grapefruit and □, orange, respectively). Each data point is the mean of $n = 6$ leaf measurements. Vertical bars indicate $\pm$ SD.

of mature leaves even though there was a significant quadratic relationship between A_{CO_2} and N. Photosynthetic N use efficiency (PNUE = A_{CO_2} /leaf N) decreased with increased N concentration similarly in young and old leaves (Fig. 2d). Young leaves had lower DW/a, N and A_{CO_2} but higher WUE than mature leaves (Fig. 2c). This means that young leaves had much lower transpiration rates than mature leaves. The lowest leaf N concentration on mature leaves corresponded to control and salt sprayed leaves that had not been fertilized for 6 weeks by the end of the experiment.

Fig. 4. The effect of weekly sprays of urea (11.2 g N/liter, 1.18 dS·m⁻¹ EC) (○), urea + NaCl-CaCl₂ (9.31 dS·m⁻¹ EC) (□), or water (0.24 dS·m⁻¹ EC) (▽) on leaf N and chlorophyll concentration of 'Duncan' grapefruit and 'Valencia' orange mature leaves. Each data point is the mean of $n = 6$ leaf measurements; vertical bars indicate $\pm$ SD.

Experiment 2. Total leaf Cl concentration increased linearly with the number of saline sprays (Fig. 3). By the week 3, grapefruit leaves had significantly higher ($P < 0.05$) Cl concentrations than orange leaves. Burn symptoms were visible on about 10% of the leaf area of grapefruit leaves after only two foliar applications. Again, no burn symptoms were observed on orange leaves even though in this experiment, leaf Cl reached 9.5 mmol·m⁻² by week 5.

Mean individual leaf area was 25.4 ± 4.2 cm² for grapefruit leaves and 26.2 ± 7.1 cm² for orange leaves. Leaf succulence was 16.3 ± 2.4 and 16.5 ± 2.6 mg·cm⁻² for grapefruit and orange leaves. Thus, there were no differences in average leaf area or succulence regardless of treatment.

Leaf N and total chlorophyll concentration were initially quite low, but increased similarly with the number of foliar urea sprays regardless of the salinity levels (Fig. 4). After 4 sprays with urea, foliar N concentration of grapefruit increased from about 100 to 250 mmol·m⁻² (about 2%–4% dry weight) whereas, N concentration in orange leaves increased up to about 200 mmol·m⁻². Leaf N for the three first weeks was not analyzed but was calculated from chlorophyll data using $N = 5.31(Chl) + 4.89$, $r^2 = 0.87$ for 'Duncan' grapefruit, and $N = 4.58(Chl) - 5.81$, $r^2 = 0.65$ for 'Valencia' orange leaves. Initial average chlorophyll concentration in grapefruit leaves (0.18 ± 0.05 mmol·m⁻²) was lower than in orange (0.33 ± 0.05 mmol·m⁻²) but after two sprays of urea, chlorophyll concentrations were similar in both species. Chlorophyll concentration of water sprayed orange leaves gradually decreased as N-deficiency progressed.

After two sprays with urea, A_{CO_2} in grapefruit increased 66%–77% over the N deficient controls (Fig. 5). There was no significant difference ($P > 0.05$) between urea and urea + saline treatments in either species. A_{CO_2} of urea-sprayed orange leaves did not increase whereas, control leaves decreased over time. Grapefruit leaves had higher A_{CO_2} than orange leaves and this difference became more apparent when these data were pooled and plotted against leaf N (Fig. 6, Table 4). DW/a was lower in grapefruit than orange at low N but grapefruit leaf DW/a increased more rapidly with increasing N than in orange leaves. There was no increase in A_{CO_2} at leaf N greater than about 200 mmol·m⁻² (Fig. 6b). WUE was positively related to leaf N

regardless of treatment indicating that both A_{CO_2} and transpiration increased similarly with leaf N concentration. As in Expt. 1, PNUE decreased with increased leaf N concentration (Fig. 6d).

Discussion

Young leaves, in Expt. 1., had lower Cl concentration than mature leaves because they received fewer saline sprays than mature leaves. Mature grapefruit leaves sprayed 11 times with NaCl+CaCl₂ solutions of 3.76 dS·m⁻¹ (Expt. 1) or 5 times with 9.31 dS·m⁻¹ (Expt. 2), absorbed sufficient Cl to reach 7 mmol m⁻² and had visible leaf injury. It is clear that the higher salt concentration in the spray solution in Expt. 2, resulted in higher Cl concentration in leaves than in Expt. 1. Our studies show that 'Valencia' orange may be less sensitive to foliar saline sprays than 'Duncan' grapefruit (Fig. 3). Whether this difference was due to differences in uptake characteristics of the leaves is not known. Other studies have shown that 'Valencia' orange is relatively less sensitive than other citrus cultivars to chloride taken up from soil (Lloyd et al., 1987; Peynado and Young, 1969).

Fig. 5. The effect of weekly sprays of urea (11.2 g N/liter, 1.18 dS·m⁻¹ EC) (○), urea + NaCl-CaCl₂ (9.31 dS·m⁻¹ EC) (□), or water (0.24 dS·m⁻¹ EC) (▽) on net CO₂ assimilation (A_{CO_2}) of 'Duncan' grapefruit and 'Valencia' orange mature leaves. Each data point is the mean of $n = 6$ leaf measurements; vertical bars indicate $\pm$ SD.

There was no increase in A_{CO_2} at leaf N greater than about 200 mmol·m⁻² regardless of whether leaf N exceeded 200 mmol·m⁻² for a long period of time (Expt. 1) or whether leaf N had recently exceeded 200 mmol·m⁻² (Expt. 2). Thus as N increased, PNUE decreased both experiments. A major difference between the two experiments was the history of leaf N. Leaves in Expt. 1 had high N levels before spray treatments, whereas leaves in Expt. 2 were N deficient before spray treatment. Even at comparable N levels, A_{CO_2} rates of mature leaves from Expt. 1 were higher than in Expt. 2. It is possible that more N was associated with photosynthetic processes and contributed to higher leaf DW in Expt. 1 than in Expt. 2. This idea is supported by the somewhat higher DW/a in Expt. 1 than in Expt. 2. A higher DW/a could have contributed to the higher A_{CO_2} and PNUE values which were about 2-fold higher for leaves in Expt. 1 than those of Expt. 2.

Net gas exchange of remaining green leaf area with high N, was affected very little by saline spray solutions that severely burned the leaves (Fig. 1.) Foliar sprays of CaCl₂ have reduced gs in apple leaves (Swietlik et al., 1984) in contrast to our results for citrus leaves. Swietlik and Miller (1987) showed that sprays of CaCl₂ on greenhouse-grown apple trees resulted in only a temporary (2-day) reduction in gs. They showed that application of CaCl₂ produced less consistent gs reductions than others salts and speculated that the development of a thick cuticle on leaves might be expected to further diminish response to CaCl₂ foliar sprays. Our data support

Fig. 6. The relationship of dry weight per leaf area (DW/a) (A), net CO₂ assimilation rate (A_{CO_2}) (B), water use efficiency (WUE) (C), and photosynthetic N use efficiency (PNUE) (D) to the leaf N concentration of mature 'Duncan' grapefruit leaves (●) and 'Valencia' orange leaves (■). Each data point is the mean of $n = 6$ leaf measurements. Regression equations and coefficient of determination are presented in Table 3.

that speculation in that citrus leaves have two-fold thicker cuticle than apple leaves (Leece, 1976).

High leaf Cl levels from foliar sprays, should have been sufficient to decrease CO₂ assimilation rates as previously reported for citrus when high salinity was in the irrigation water (García-Legaz et al., 1993; Lloyd et al., 1987). We found no effect of high leaf Cl on A_{CO_2} however, when Cl were sprayed onto leaves. Chloride concentrations in necrotic portions of leaves could have been slightly higher than in the green portions that were used for measurements of gas exchange. This possibility probably did not affect our interpretation of the lack of Cl effects on net gas exchange, however, because high Cl concentrations in 'Valencia' leaves with no burn symptoms did not reduce A_{CO_2} (Figs. 3 and 5). We did not evaluate the effect of leaf area loss on whole plant A_{CO_2}

Table 4. Regression coefficients (β_n) and coefficient of determination (r^2) for relationships between dry weight per leaf area (DW/a), CO₂ assimilation rate (A_{CO_2}), water use efficiency (WUE), and photosynthetic N use efficiency (PNUE) (dependent variable, y) and the leaf N concentration (independent variable, x) of 'Duncan' grapefruit and 'Valencia' orange leaves. Data from Expt. 2, Figs. 6 and 7.

y vs. x	Cultivar	β_0	β_1 (x)	β_2 (x ²)	r^2
DW/a vs. N	Duncan	53.15	0.09	---	0.93
	Valencia	67.31	0.03	---	0.30
A_{CO_2} vs. N	Duncan	0.82	0.03	-4.6×10^{-5}	0.89
	Valencia	-25	0.04	-8.9×10^{-5}	0.84
WUE vs. N	Duncan	1	0.01	---	0.78
	Valencia	1.11	0.01	---	0.60
PNUE vs. N	Duncan	0.06	-3.2×10^{-4}	6.9×10^{-7}	0.73
	Valencia	0.03	-6.7×10^{-5}	---	0.54

but total A_{CO_2} per plant, could have been reduced by the salinity-induced loss of leaf area. Some compensation by remaining leaf area can occur (Layne and Flore, 1992; Syvertsen and McCoy, 1985) so whole plant A_{CO_2} would not necessarily have been reduced in proportion to the amount of leaf area loss.

High N and total chlorophyll concentration of leaves sprayed with saline+urea solutions occurred in conjunction with the increase of leaf Cl (Figs. 3 and 4). Gas exchange responses to leaf N were not modified by increases of chloride in leaf tissues (Fig. 6). Thus, there was no evidence that salinity in the spray solution reduced foliar uptake of nitrogen or reduced chlorophyll concentration in remaining green tissues. These results differ from other salinity stress studies in which there was a reduction of total chlorophyll when leaf chloride concentration increased (Khan et al., 1994; Nieves et al., 1991). Again, our foliar application study differs from previous studies where N and Cl were taken up from soil-applied irrigation water. Our results imply that Cl effects on chlorophyll and A_{CO_2} from foliar-applied salts to well watered seedlings may not be as serious as when the root system is exposed to salinity stress. Future studies should separate the potential physiological differences between foliar and root uptake of Cl and N.

This study evaluated the potentially negative effects of salt spray solutions along with the potentially beneficial effects of urea sprays on citrus leaves with sufficient or deficient N. Even relatively high concentrations of salts in the spray solution did not reduce uptake of foliar-applied N or rates of A_{CO_2} . Repeated spray applications of urea to well-nourished plants did not increase A_{CO_2} . Three weekly sprays increased N from 100 mmol·m⁻² to greater than 200 mmol·m⁻², which was sufficiently high to support high rates of A_{CO_2} . It is not yet possible, however, to extrapolate these results to field condition where leaf N levels are generally lower than those in this study.

Literature Cited

- Beckett, T.H. and E.W. Stoller. 1991. Effects of methylammonium and urea ammonium nitrate on foliar uptake of thifensulfuron in velvetleaf (*Abutilon theophrasti*). *Weed Sci.* 39:333–338.
- Behboudian, M.H., E. Törökfalvy, and R.R. Walker. 1986. Effects of salinity on ionic content, water relations, and gas exchange parameters in some citrus scion-rootstock combinations. *Scientia Hort.* 28:105–116.
- Eaton, F.M. and R.B. Harding. 1959. Foliar uptake of salt constituents of water by citrus plants during intermittent sprinkling and immersion. *Plant Physiol.* 33:22–26.
- Embleton, T.W., M. Matsumura, L.H. Stolzy, D.A. Devitt, W.W. Jones, R. El-Motaium, and L.L. Summers. 1986. Citrus nitrogen fertilizer management, groundwater pollution, soil salinity, and nitrogen balance. *Appl. Agr. Res.* 1(1):57–64.
- Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78:9–19.
- García-Legaz, M.F., J.M. Ortiz, A. García-Lidón, and A. Cerdá. 1993. Effect of salinity on growth, ion content and CO₂ assimilation rate in lemon varieties on different rootstocks. *Physiol. Plant.* 89:427–432.
- Gratani, L. 1993. Response to microclimate of morphological leaf attributes, photosynthetic, and water relations of evergreen sclerophyllous shrub species. *Photosynthetica* 29:573–582.
- Hanlon, E.A., T.A. Obreza, A.K. Alva. 1995. Tissue and soil analysis, p. 13–16. In: D.P.H. Tucker, A.K. Alva, L.K. Jackson, and T.A. Wheaton (eds.). *Nutrition of Florida citrus trees*. Univ. of Florida, Gainesville.
- Kannan, S. 1980. Mechanisms of foliar uptake of plant nutrients: Accomplishments and prospects. *J. Plant Nutr.* 2:717–735.
- Khan, M.G., M. Silberbush, and S.H. Lips. 1994. Physiological studies on salinity and nitrogen interaction in alfalfa. II. Photosynthesis and transpiration. *J. Plant Nutr.* 17:669–682.
- Layne, D.R., and J.A. Flore (1992). Photosynthetic compensation to partial leaf area reduction in sour cherry. *J. Amer. Soc. Hort. Sci.* 117:279–286.
- Lea-Cox, J.D. and J.P. Syvertsen. 1995. Nitrogen uptake by citrus leaves. *J. Amer. Soc. Hort. Sci.* 120:505–509.
- Leece, D.R. (1976). Composition and ultrastructure of leaf cuticles from fruit trees, relative to differential foliar absorption. *Austral. J. Plant Physiol.* 3:833–847.
- Lloyd, J. and H. Howie. 1989. Salinity, stomatal responses, and whole-tree hydraulic conductivity of orchard 'Washington Navel' orange, *Citrus sinensis* (L.) Osbeck. *Austral. J. Plant Physiol.* 16:169–179.
- Lloyd, J., J.P. Syvertsen, P.E., and Kriedemann. 1987. Salinity effects on leaf water relations and gas exchange of 'Valencia' orange, *Citrus sinensis* (L.) Osbeck, on rootstocks with different salt exclusion characteristics. *Austral. J. Plant Physiol.* 14: 605–617.
- Maas, E.V. 1993. Salinity and citriculture. *Tree Physiol.* 12:195–216.
- Maas, E.V. and J.G. Hoffman. 1977. Crop salt tolerance-current assessment. *J. Irrig. Drain. Div. Amer. Soc. Civ. Eng.* 103(IR2):115–134.
- Nieves, M., A. Cerdá, and M. Botella. 1991. Salt tolerance of two lemon scions measured by leaf chloride and sodium accumulation. *J. Plant Nutr.* 14:623–636.
- Peinado, A. and R. Young. 1969. Relation of salt tolerance to cold hardiness of 'Redblush' grapefruit and 'Valencia' orange trees on various rootstocks. *Proc. 1st Intl. Citrus Symp.* 3:1793–1802.
- Porra, R.J., W.A. Thompson, and P.E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* 975: 384–394.
- Shalhevet, J. and Y. Levy. 1990. Citrus Trees, p. 951–986. In: *Irrigation of agricultural crops-Agronomy*. Monogr. no. 30. ASA-CSSA, Madison, Wis.
- Sinclair, T.R. 1990. Nitrogen influence on the physiology of crop yield., p. 41–55. In: R. Rabbinge, J. Goudriaan, H. Van Keulen, F.W.T. Penning de Vries, and H.H. van Laar (eds.). *Theoretical production ecology: Reflections and prospect*. Pudoc, Wageningen.
- Syvertsen, J.P. 1984. Light acclimation in citrus leaves. II. CO₂ assimilation and light, water, and nitrogen use efficiency. *J. Amer. Soc. Hort. Sci.* 109:812–817.
- Syvertsen, J.P. and C.W. McCoy. 1985. Leaf feeding injury to citrus by root weevil adults: Leaf area, photosynthesis, and water use efficiency. *Florida Entomol.* 68:386–393.
- Syvertsen, J.P. 1987. Nitrogen content and CO₂ assimilation characteristics of citrus leaves. *HortScience* 22:289–291.
- Syvertsen, J.P., B. Boman, and D.P.H. Tucker. 1989. Salinity in Florida citrus production. *Proc. Fla. State. Hort. Soc.* 102:61–63.
- Swietlik, D., J.A. Bunce, and S.S. Miller. 1984. Effect of foliar application of mineral nutrients on stomatal aperture and photosynthesis in apple seedlings. *J. Amer. Soc. Hort. Sci.* 109:306–312.
- Swietlik, D., and S.S. Miller. 1987. Effect of foliar-applied calcium and chlorine on stomatal conductance in apple leaves. *HortScience* 22:626–628.
- Tennessen, D.J., E.L. Singsaas, and T.D. Sharkey. 1994. Light-emitting diodes as a light source for photosynthesis research. *Photosyn. Res.* 39:85–92.
- Wittwer, S.H., M.J. Bukovac, and H.B. Tukey. 1963. Advances in foliar feeding of plant nutrient, p. 429–455. In: M.H. McVickar, G.L. Bridger, and L.B. Nelson (eds.). *Fertilizer technology and usage*. Amer. Soc. Agron., Madison, Wis.